VOLUME 36 DECEMBER 1958 NUMBER 6

Canadian Journal of Zoology

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF ZOOLOGY

(Formerly Section D, Canadian Journal of Research)

Under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research, the National Research Council issues THE CANADIAN JOURNAL OF ZOOLOGY and five other journals devoted to the publication, in English or French, of the results of original scientific research. Matters of general policy concerning these journals are the responsibility of a joint Editorial Board consisting of: members representing the National Research Council of Canada; the Editors of the Journals; and members representing the Royal Society of Canada and four other scientific societies.

Manuscripts for publication should be submitted to Dr. T. W. M. Cameron, Editor, Canadian Journal of Zoology, Institute of Parasitology, Macdonald College, P.Q., Canada. (For instructions on preparation of copy, see **Notes to Contributors** (inside back cover))

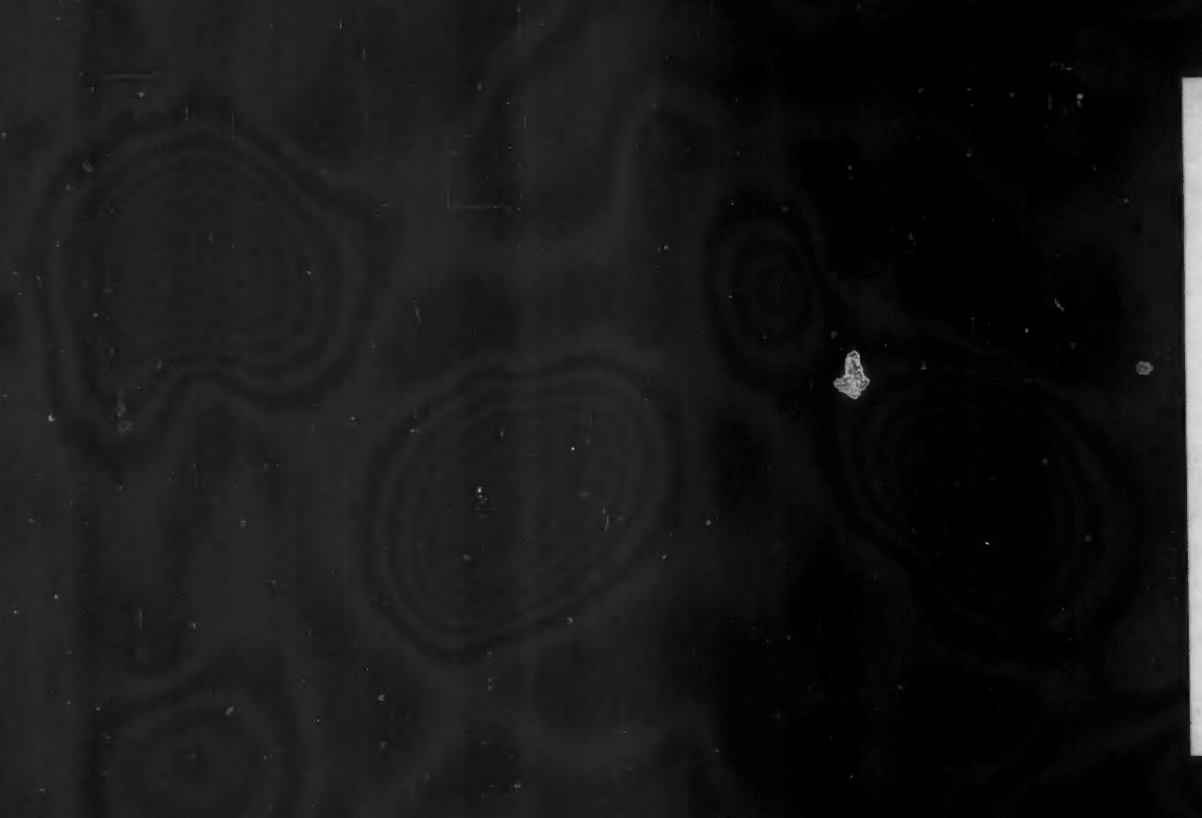
Proof, correspondence concerning proof, and orders for reprints should be sent to the Manager, Editorial Office (Research Journals), Division of Administration and Awards, National Research Council, Ottawa 2, Canada.

Subscriptions, renewals, requests for single or back numbers, and all remittances should be sent to Division of Administration and Awards, National Research Council, Ottawa 2, Canada. Remittances should be made payable to the Receiver General of Canada, credit National Research Council.

The journals published, frequency of publication, and prices are:

Canadian Journal of Biochemistry and Physiology	Monthly	$3.00 a year
Canadian Journal of Botany	Bimonthly	$4.00
Canadian Journal of Chemistry	Monthly	$5.00
Canadian Journal of Microbiology	Bimonthly	$3.00
Canadian Journal of Physics	Monthly	$4.00
Canadian Journal of Zoology	Bimonthly	$3.00

The price of regular single numbers of all journals is 75 cents.



CORRECTION

Can. J. Zool. 36, 307 (1958). The following information should be inserted in Table I between the species *Eriosoma lanigerum* (Hausm.) and *Rhopalosiphum maidis* (Fitch), under Aphididae:

Macrosiphoniella millefolii (DeGeer)	*Achillea millefolium* L.	17/8/57	+			
Macrosiphum solanifolii (Ashm.)	*S. tuberosum* L.	10/7/56	+	+	+	
Macrosiphum solanifolii (Ashm.)	*Rosa* sp.	12/7/57	−			
Melaphis rhois (Fitch)	*Rhus typhina* L.	18/8/56		−		
Myzus cerasi (Fab.)	*Prunus pennsylvanica* L.	9/9/56	−	−	−	−
Myzus persicae (Sulz.)	*S. tuberosum* L.	−/7/55 and −/7/56	+		+	
Myzus persicae (Sulz.)	*Brassica napobrassica* Mill.	−/8/56	+	+	+	
Periphyllus negundinis (Thomas)	*Acer negundo* L.	11/8/56	+			
Phorodon humuli (Schrank)	*Prunus nigra* L.	12/6/56	+	−	+	−
Phorodon humuli (Schrank)	*Humulus* sp.	12/8/57	−			
Prociphilus tessellata (Fitch) (*Pterocomma populifoliae* near this species (Fitch))	*Acer negundo* L.	10/9/57		−		
	Salix sp.	8/8/57	+			
Pterocomma smithiae (Monell)	*Salix* sp.	8/8/57	+			

Canadian Journal of Zoology

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 36 DECEMBER 1958 NUMBER 6

IMPREGNATION OF HETERODERA TRIFOLII BY MALES OF H. SCHACHTII (NEMATODA : HETERODERIDAE)[1]

ROLAND H. MULVEY[2]

Abstract

A parthenogenetic nematode, *Heterodera trifolii* (Goffart, 1932) Raski and Hart, 1953, was impregnated by a bisexual nematode, *H. schachtii* Schmidt, 1871, in mixed cultures of these two cyst-forming species. No males occurred among several hundred offspring.

Introduction

Until 1953 the clover-cyst nematode, *Heterodera trifolii* (Goffart, 1932) Raski and Hart, 1953, was considered a variety of the sugar-beet nematode, *H. schachtii* Schmidt, 1871, but is now recognized as a distinct species. These two species of cyst-forming nematodes are markedly different both morphologically and cytologically. *H. schachtii* is a bisexual nematode in which males are common, whereas *H. trifolii* reproduces parthenogenetically (4) and only in a few instances have males been found (1).

This is a report on a study on whether males of *H. schachtii* might impregnate *H. trifolii*.

Materials and Methods

Whether impregnation occurred was tested in mass cultures and in cultures from single cysts. Cysts for cultures of the sugar-beet nematode were obtained from red beet roots and those for the clover-cyst nematode from white Dutch clover roots. All plants were grown in a greenhouse at Ottawa.

Mass cultures were used as follows. Five-inch pots were nearly filled with the following soil mixtures: (*a*) sterilized soil plus soil heavily infested with the clover-cyst nematode; (*b*) sterilized soil plus soil heavily infested with the sugar-beet nematode; (*c*) a mixture of (*a*) and (*b*).

Red beet and white Dutch clover seeds were planted in all pots. Pots in series (*a*) and (*b*) were used as checks.

Cultures from single cysts were used as follows: A box was partly filled with a mixture of sterilized sand and soil heavily infested with the clover-cyst nematode. After a few days white Dutch clover seedlings were transplanted into this box. A second box was partly filled with a mixture of sterilized

[1]Manuscript received July 7, 1958.
Contribution No. 3827, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

[2]Nematode Section, Entomology Laboratory, Ottawa.

[The previous number of Can. J. Zoology (36, 629–838) was issued October 17, 1958.]

sand and soil heavily infested with the sugar-beet nematode. A few days later red beet seedlings were transplanted into this box. After 2 days, during which the second-stage larvae had entered the plant roots, the seedlings were removed and washed free of soil debris and one plant of each kind was replanted in each of twenty-five 5-in. pots containing sterilized soil compost.

Thirty days after replanting, both white Dutch clover and red beet plants were examined for the presence of white female nematodes on the roots. Each plant was carefully washed before examination by immersing the roots in water. The roots were then examined under the microscope and live white females, which were attached to the roots, were removed and immediately placed in Carnoy's fluid. Slide material was prepared for study by the squash technique (5) and in the manner described by Mulvey (2). Each *H. trifolii* female found containing sperm was carefully checked for the number of chromosomes in the oöcytes. *H. schachtii* and *H. trifolii* differ in chromosome number (3, 4).

Results

Mass Cultures

In the mixed culture the females from the red beet plants had well-developed spermathecae, most of which had numerous tailless sperm, and 12 of the 45 females from the white Dutch clover roots showed sperm in their reproductive systems.

In the culture of the clover-cyst nematode, only the white Dutch clover was attacked. Examination of the clover roots and screening of the soil showed that no males were present, and sperm was not found in the reproductive systems of the many females examined. In the culture of the sugar-beet nematode only the red beet was attacked. Both males and females were present, and sperm was abundant in the spermathecae of the females.

Cultures from Single Cysts

Many of the females from red beet roots were fertilized. Six of the 62 females from the white Dutch clover roots had sperm in their reproductive tracts. The chromosome number in the impregnated females was the same as that reported by Mulvey (4) for *H. trifolii*.

These results indicate that *H. trifolii* females may be impregnated by *H. schachtii* males in mixed populations of the two species. However, limited tests have revealed that no males are produced in the several hundred offspring of several impregnated females.

Acknowledgment

The author is indebted to Mr. A. L. Sauve, greenhouseman, for his help in propagating and maintaining the plants during the study.

References

1. FRANKLIN, M. T. The cyst-forming species of *Heterodera*. Wilding & Son Ltd., Shrewsbury, England. 1951.
2. MULVEY, R. H. Oogenesis in several free-living and plant-parasitic nematodes. Can. J. Zool. **33**, 295–310 (1955).
3. MULVEY, R. H. Chromosome number in the sugar-beet nematode, *Heterodera schachtii* Schmidt. Nature, **180**, 1212–1213 (1957).
4. MULVEY, R. H. Parthenogenesis in a cyst-forming nematode, *Heterodera trifolii* (Nematoda : Heteroderidae). Can. J. Zool. **36**, 91–93 (1958).
5. SMITH, S. G. Techniques for the study of insect chromosomes. Can. Entomologist, **75**, 21–34 (1934).

NEST ATTENTIVITY OF LINCOLN'S SPARROW DETERMINED USING THERMISTOR BRIDGE[1]

J. Murray Speirs and R. Andoff

Abstract

A new attentivity recorder is described for use in studying the incubation rhythm of nesting birds. This was used in a nest of Lincoln's sparrow, *Melospiza lincolnii*. On the fifth day of incubation 10 attentive periods averaged 20.4 minutes while 11 inattentive periods averaged 6.9 minutes. The incubation period was found to be 13 days (time from laying of last egg to hatching of last egg).

Introduction

Kendeigh and Baldwin (1930 (4, p. 473)) have described an attentivity recorder used in their classic study of the house wren. This "itograph" is particularly suitable in studying the periods on and off the nest of species which, like the house wren, nest in boxes and must pass to and from the nest by a definite entrance. A pivoting beam is mounted with electrical contacts inside and outside the entrance hole and the arrivals and departures of the bird are recorded on a moving strip of paper at a distance from the nest. Hahn (1937 (3, pp. 223 and 234)) and Fant (1953 (2, p. 323)) have described variants of the itograph principle for use in ground- and tree-nesting species. The itograph is a relatively inexpensive and portable instrument and is probably the best for studies of abundant species which have little fear of man. Another instrument described by Baldwin and Kendeigh (1932 (1, p. 19)) consists of a copper–constantan thermocouple which may be inserted very inconspicuously into the nest lining. It has leads going to a recording potentiometer in a nearby laboratory. This instrument has two great advantages: (1) the thermocouple which is inserted into the nest is so inconspicuous that there is practically no disturbance of the normal nesting rhythm once the element has been installed; and (2) the potentiometer record can be calibrated to give readings of actual nest temperatures if so desired. The big disadvantages are the original cost of the potentiometer and its lack of portability, so that studies with this equipment are limited to the vicinity of permanent laboratories with adequate financial support.

The Lincoln's sparrow, *Melospiza lincolnii*, is a secretive, retiring bird. Very little has been published about its nest life. Its incubation period was unknown and nothing has been published about its attentivity rhythm at the nest. The closely related song sparrow, *Melospiza melodia*, on the other hand is one of the most thoroughly studied of North American birds (Nice, 1937 (5) and 1943 (6)).

[1]Manuscript received May 3, 1958.
Contribution from the Department of Zoology, University of Toronto, Toronto, Ontario.

In order to study the nest life of the Lincoln's sparrow it seemed desirable to obtain a nest recorder with the portability of the "itograph" and yet with an inconspicuous sensitive element in the nest. The junior author undertook to devise such a recorder. Piezoelectric crystals under the nest were considered but rejected as interfering too much with the nest. A device similar to the proximity fuse that powered the booby traps of ill repute in recent conflicts was brought to the laboratory test stage successfully so that the mere change in electrical capacity of the bird going on the nest would actuate a relay and pen on paper. However this capacity relay proved unreliable under field conditions with large changes in temperature and moisture. Thermistor bridges were then tried. The 1956 model required a storage battery and used radio tubes in the bridge circuit. The apparatus proved too heavy for convenient field use and the batteries required recharging too often when tubes were used. In the 1957 model the tubes were replaced with transistors and this allowed the use of small dry cells.

Description of the Thermistor Bridge Recorder

The instrument finally developed provided a reasonably small, light, and accurate means of determining nesting periods. Two matched thermistors were employed. One was inserted into the lining of the bird's nest so that it would be heated by the incubating bird and would cool off when she left the nest. The other thermistor was left just outside the nest. When the bird was off the nest the two thermistors should be at the same temperature while when the bird is on the nest the thermistor probe in the nest would be warmer by several degrees. The thermistors used had little thermal inertia and followed the temperature changes in the nest fairly quickly (usually within 5 seconds upon application of heat and within 15 seconds after cessation of heating).

The equipment used to record the temperature difference between the two thermistors consisted of the following main components:

(1) two matched thermistors, one to be inserted into the nest (the nest probe) and the other left outside it (the compensator probe);
(2) a temperature-compensated Wheatstone bridge circuit;
(3) a bridge amplifier and relay;
(4) a pen-actuating mechanism;
(5) a chart drive;
(6) a 25-foot cable for the probes;
(7) a plywood carrying case to house the equipment;
(8) dry cells to operate the electrical components.

Figure 1 shows the electrical circuits. The bridge is a conventional Wheatstone circuit in which the thermistors Tr1 and Tr2 form adjacent arms. The variable resistance (sensitivity control) R3 and the fixed resistances R1 and R2 are operated in unbalance to supply a preset bias voltage to the amplifier transistors V1 and V2.

When Tr1 (nest probe) is heated, this tends to balance the bridge, reducing the bias voltage on V1 and V2, causing them to conduct. This pulls in the relay Rl1 which completes the pen actuator circuit. A pair of 2N107 transistors were used in parallel to provide sufficient collector current to operate Rl1.

The compensator probe corrects for changes in the ambient temperature of the air. If the air temperature drops, the compensator maintains the same relative differential, hence the bridge remains stable. Western Electric type 14B thermistors were used because of their small size (glass rods about 3 in. long and $\frac{1}{16}$ in. in diameter).

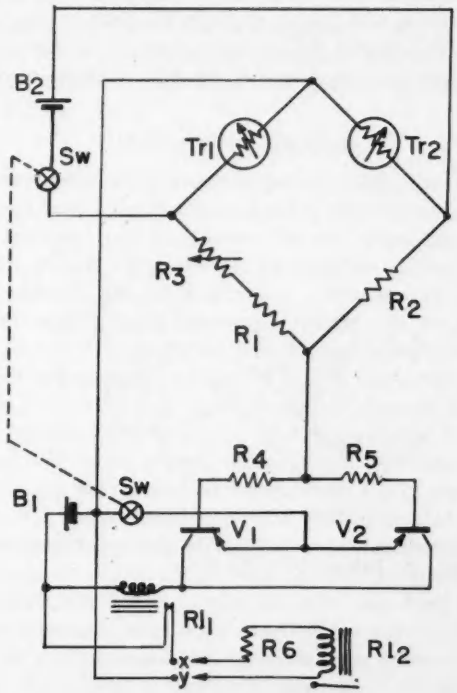


FIG. 1. Electrical circuit for thermistor bridge. B1. 9-volt battery. B2. 3-volt battery. R1. 1000-ohm resistance. R2. 2700-ohm resistance. R3. Variable resistance (to 2000 ohms). R4, R5. 3300-ohm resistances. R6. 330-ohm resistance. Tr1, Tr2. Thermistors (2000 ohms at 25° C, resistance decreasing 3.9% per rise of one Centigrade degree). V1, V2. Transistors (2N107). See text for operational detail.

The pen actuator consists of a modified relay E and a pen carriage C illustrated in Fig. 2. Figure 3 is a vertical section of the pen carriage. The pen carriage rotates in a small ball bearing E. Its shaft extends through the bearing to a small chamber G containing silicone fluid. A vane F attached to the shaft is damped by the fluid in the chamber to prevent the relay from throwing the pen violently across the chart. Silicone fluid was also used on

the knife-edge pen bearing B to reduce pen kick further. The pen itself was a standard Esterline-Angus 8510 capillary feed pen, fed from a well D in the pen carriage. Extra ink was carried in a dropper bottle in the carrying case.

Power for the bridge was supplied by two 1.5-volt 'D' cells. The amplifier and pen actuator were powered by two 4.5-volt dry cells. The record chart was mounted on a plastic disk rotated by a 24-hour, heavy-duty clockwork motor.

Some experimentation is necessary to achieve the optimum setting of the sensitivity control. The control is advanced until a deflection is obtained of the pen on the chart: it is then backed off slightly. The unit then operates at maximum sensitivity and this is probably the best setting for small species. For larger species the control should be backed off farther to reduce the sensitivity and prevent false readings induced by sudden environmental temperature changes.

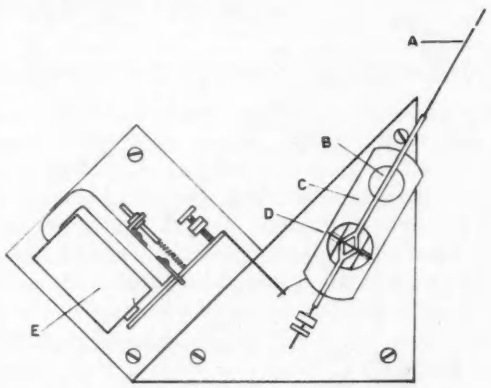


FIG. 2. Plan of pen actuator. A. Capillary pen. B. Inkwell. C. Pen carriage. D. Bearings for pen. E. Relay (Rl2 in Fig. 1).

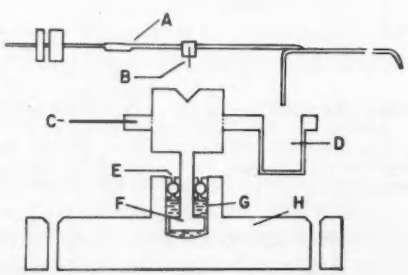


FIG. 3. Vertical section of pen carriage. A. Capillary pen. B. Knife edge which fits into V bearing below it. C. Operating pin. D. Inkwell into which capillary pen dips when knife edge is lowered into V bearing. E. Ball bearing in which pen carriage rotates when actuated by relay which is jointed to operating pin. F. Vane damped by silicone fluid in chamber G. See text for operational detail.

Incubation Period

Doris H. Speirs found a Lincoln's sparrow nest on May 31, 1957, by the side of the entrance road leading into the Dorion Trout Rearing Station (Stirling Township, Thunder Bay District, Ontario). On June 1 the first egg was laid before 7.30 a.m. (and after 7.15 p.m., May 31); the second was laid after 8.30 p.m., June 1, and before 5.30 a.m. on June 2; the third on June 3 before 6.55 a.m.; and the final egg after 7.35 p.m. on June 3 and before 7.20 a.m. on June 4. Each egg, as discovered in the nest, was marked using a grass blade dipped into India ink. Neil Atkinson noted four eggs in the nest at 10.00 a.m. on June 16. At 4.25 p.m. and again at 8.55 p.m. the nest contained three eggs and one young (from the first egg laid). On June 17 at 7.05 a.m. the nest contained three young and the unhatched fourth egg. By 12.35 p.m. the fourth egg had hatched. We were thus enabled to determine the incubation period as 13 days.

Attentivity Rhythm

The attentivity rhythm at this nest on June 8, 1957 (the fifth day of incubation of the final egg laid), was determined using the thermistor bridge recorder. The bird apparently did not flush from the nest when the record chart was changed at 7.30 a.m. It remained on the nest for 2 hours and 2 minutes, until 9.32 a.m. when it left for 5 minutes. It returned to the nest for 21 minutes, then left again for 6 minutes. Before the recording pen ceased functioning at 2.12 p.m. 11 inattentive periods (bird off the nest) were recorded: these averaged 6.9 minutes with a range from 2 to 15 minutes. There were 10 attentive periods after 9.32 a.m. averaging 20.4 minutes with a range from 17 minutes to 40 minutes, with one very brief period when the bird apparently came to nest and left again almost immediately. During the period from 9.32 a.m. to 2.12 p.m. the incubating bird spent 75% of its time on the nest and 25% off. It was a clear, mild day. On the morning of June 11, an overcast, mild day, attentive periods of 25 minutes, 54 minutes, and 18 minutes were broken by inattentive periods of 3 minutes and 1 minute. The increased attentivity on this eighth day of incubation may have been influenced by the overcast weather (temperatures were about the same on the two days) or may have been an expression of the intensification of the incubation habit. Nice (1937 (5, p. 124)) found a similar shortening of the inattentive periods in her study of the song sparrow as incubation progressed. She gives the average attentive period of the song sparrow as 20 to 30 minutes, with average inattentive period as 6 to 8 minutes, and notes the percentage of time spent on the nest, varying from 76 to 80 for four individuals and 72 for another (from data supplied by Baldwin and Kendeigh determined by thermocouple and potentiometer). The incubation rhythm found in the present study of the Lincoln's sparrow is thus very similar to Nice's findings for the song sparrow.

Acknowledgments

The authors are indebted to the National Research Council of Canada for financial assistance in developing the thermistor bridge and to Dr. R. R.

Langford of the Department of Zoology, University of Toronto, Dr. G. E. Edmund, Royal Ontario Museum, and Dr. R. W. McKay, Department of Physics, University of Toronto, for material assistance and advice. We are grateful to Dr. Edmund also for preparing the illustrations.

References

1. BALDWIN, S. P. and KENDEIGH, S. C. Physiology of the temperature of birds. Sci. Publs. Cleveland Museum Nat. Hist. **3**, i–x, 1–196 (1932).
2. FANT, R. J. A nest-recording device. Animal Ecol. **22**, 323–327 (1953).
3. HANN, H. W. Life history of the oven-bird in southern Michigan. Wilson Bull. **49**, 145–237 (1937).
4. KENDEIGH, S. C. and BALDWIN, S. P. The mechanical recording of the nesting activities of birds. Auk, **47**, 471–480 (1930).
5. NICE, M. M. Studies in the life history of the song sparrow I. Trans. Linnaean Soc. New York, **4**, i–vi, 1–247 (1937).
6. NICE, M. M. Studies in the life history of the song sparrow II. The behavior of the song sparrow and other passerines. Trans. Linnaean Soc. New York, **6**, i–viii, 1–328 (1943).

BIOLOGY OF OTODECTES CYNOTIS, THE EAR CANKER MITE OF CARNIVORES[1]

GORDON K. SWEATMAN[2]

Abstract

The life history of *Otodectes cynotis* has been observed in vitro and all stages in the cycle described. Mites from the dog, cat, fox, and ferret have been shown to be biologically and morphologically identical, and the varietal names of *canis*, *cati*, and *furonis* are invalidated. Other Carnivora also appear to be potential hosts.

Introduction

Otodectic mites generally live deep in the ear canal near the eardrum of dogs, foxes (*Vulpes fulva* and *Alopex lagopus*), cats, and ferrets (*Putorius putorius*). The mites are frequently unperceived, but heavily infested dogs and cats have displayed epileptiform spasms, sometimes as frequently as a dozen times daily (13). Lesions on the body have been observed also (5, 10). An epizootic occurred among wild ferrets in 1884 in northern France, particularly around Calais, but this host showed no symptoms even when the tympanum was destroyed (*in* Ref. 8). There is one authentic case (*in* Ref. 8) of a cat suspected of rabies which was later disproved following appropriate inoculations of the cat's tissues and discovery of a severe infestation of ear mites.

Although the names for the ear mites have been given specific rank in the past, all are currently considered as varieties (*canis*, *cati*, and *furonis*) of the single species *O. cynotis*. This is based on limited experimental data from which the mites in each host family were considered biologically distinct even though morphologically indistinguishable. Morphological data have been restricted to the adult stages of the mite, hence the present paper includes a description of all stages in the mite's life cycle. This is followed by observations on the biology of the mite from one host, and then mites from all hosts are compared morphologically and biologically using the in vitro technique described (15) previously for *Chorioptes bovis* to ascertain if there are really any differences between them.

I. Morphology and Life History

A. Description of Stages

Otodectic mange mites are obligatory, non-burrowing mites that pass all stages in the ear or less frequently on the body of the host. A single cycle of the mite includes the egg, larva, protonymph, deutonymph, and adult.

[1]Manuscript received April 1, 1958.
Contribution from the Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, in co-operation with the Institute of Parasitology, Macdonald College P.O., Que., Canada.

[2]Former address: Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, Montreal Area Branch Laboratory, Macdonald College. Present address: Hydatid Research Unit, Medical School, University of Otago, Dunedin, New Zealand.

The adult male and ovigerous female are morphologically distinct but no immature stage shows sexual dimorphism. Since the sex of the deutonymph cannot be determined, the term "pubescent female" which is used in some previous publications is suppressed in favor of simply "deutonymph". Tracheal tubes occur immediately below the integument of all stages except the egg, and these show no consistent pattern even within the same stage.

In the following description, the structural nomenclature is the same as that used previously for *C. bovis* (15), and measurements are given in microns with the mean and standard deviation of individuals (measured 15) in the first and second parentheses respectively.

Larva (Fig. 1)

An oval mite with moderately short front legs and quite short hind legs. It is grayish brown and soft-bodied. No sexual dimorphism was observed.

Dorsum

Length 138–224 (179) (26) and width 103–159 (136) (15). Integument finely striated except for a sclerotized triangular propodosomal plate. Near, but not attached to, the posterior corners of the plate is a pair of short propodosomal plate setae. Posterolaterally to the propodosomal plate setae is a pair of large dorsal setae, and in addition to these there are six pairs (*Chorioptes bovis* has seven) of idiosomal setae.

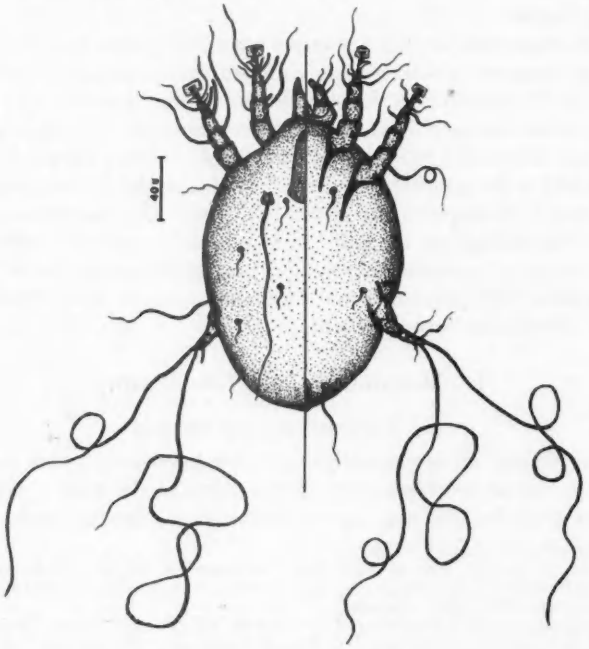


FIG. 1. Dorsoventral view of larva.

Venter

The integument is finely striated with four pairs of setae, including one long pair of terminal opisthosomal setae.

Legs

Legs I and II with six articles each, and of about the same length, being 77–105 (91) (8) and 77–105 (92) (8) respectively.* Leg III measuring 55–72 (63) (5)† and with six weak articles. Coxae of all legs specialized as apodemata. Trochanters with no setae; femurs I and II with a single seta each; genu II with two setae and genu I with two setae and a short rod; tibiae I and II with a seta and rod each, the latter situated anterodorsally; tarsus I with seven setae, a rod, and a pit, and tarsus II with five fairly long setae and a short one, the latter occurring just anterior to a moderately short rod in the proximodorsal position. Tarsi I and II each with a single claw and a short unjointed pedunculated caruncle. Pretarsi absent.

Leg III possesses a fairly long seta on the subdistal article, and a rod, two short setae, and terminally two extremely long whiplike setae of which the posterior is longer than the anterior.

Gnathosoma

Chelicerae dorsal and measure 37–52 (42) (6) long, chelate with the movable digit articulating in a cleft of the fixed digit, and each digit with three hooked teeth. Same as that figured for *Chorioptes bovis* (15).

Pedipalpi located at lateroventral surface of gnathosoma. Possess three pairs of articles which curve medially giving the gnathosoma a blunt appearance. Middle article with two setae and distal article with one seta. The hypostome possesses one pair of fairly long, centrally located setae. The gnathosoma is virtually the same in all stages, except for increase in relative size.

Protonymph (Fig. 2)

This stage shows no sexual dimorphism. The fourth leg occurs, but is inconspicuous. Additional setae occur beyond those seen on the larva.

Dorsum

Length 187–293 (230) (29) and width 150–214 (179) (18). Possesses propodosomal plate and same setae as larva with one additional pair of short idiosomal setae.

Venter

In addition to the four pairs of setae on the larva, there is one pair of metapodosomal setae and five pairs of short opisthosomal setae.

Legs

Leg I 97–127 (115) (9); leg II 106–122 (114) (9); leg III 60–79 (73) (5); and leg IV 5–8 (6) (1). In addition to the details described for the larva, genu II has a short rod located immediately anterior to the long dorsal seta;

*Measured from the coxa along the anterodorsal border. Does not include caruncle.
†Measurement of the articulating part of the leg along the outer surface.

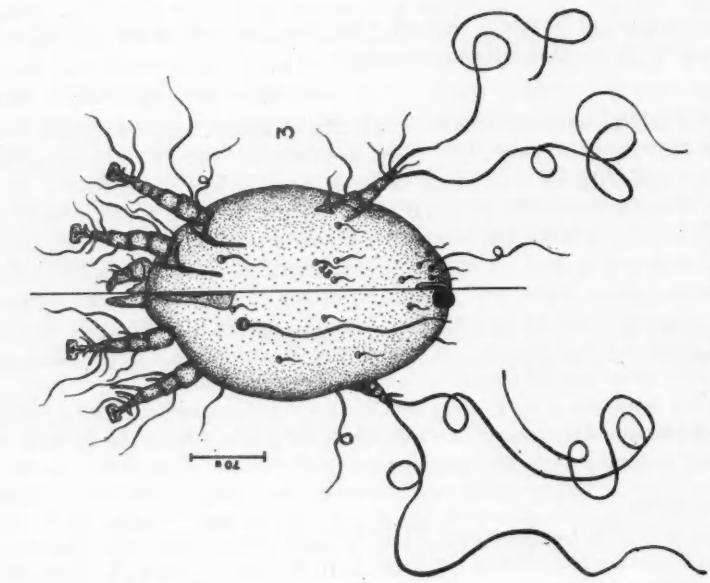


FIG. 3. Dorsoventral view of deutonymph.

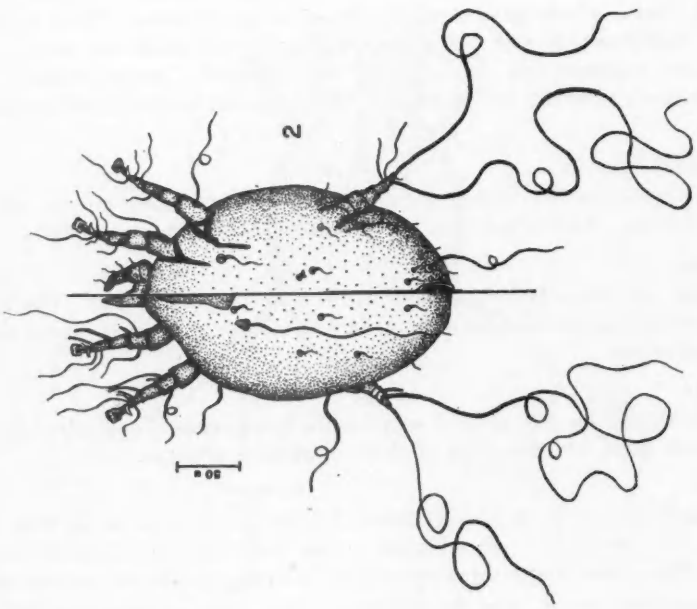


FIG. 2. Dorsoventral view of protonymph.

and the rod on leg III has moved from the distal (as on larva) to the subdistal article. Leg IV has two weak articles, the last of which has a short seta and a moderately long terminal seta.

Gnathosoma

Same as for larva, except for increase in size. Chelicerae 43–55 (48) (4).

Deutonymph (Fig. 3)

This stage shows no sexual dimorphism, and does not possess the fourth pair of legs. A pair of attachment suckers occurs in the posterodorsal position.

Dorsum

Length 225–356 (296) (37) and width 151–262 (217) (34). Posterior attachment suckers occur, but the setal pattern is identical with the protonymph.

Venter

There are two additional pairs of metapodosomal setae besides those noted for the protonymph.

Legs

Leg I 121–142 (129) (8); leg II 122–143 (129) (8); and leg III 72–82 (79) (5). Trochanters I and II have now acquired a long seta each, and tarsus I has an additional anterior rod. Other features are identical with protonymph.

Gnathosoma

As for preceding stages. Chelicerae 55–60 (58) (2).

Adult Male (Fig. 4)

Distinct morphological changes occur between the deutonymph and adult male. Additional sclerotization occurs. Leg III is transformed from a sensory structure to an essentially ambulatory one.

Dorsum

Length 274–362 (315) (28) and width 209–296 (259) (4). Additional sclerotization occurs including a large plate that covers much of the hysterosoma. The setae on the deutonymph occur on this stage plus one pair of very long, two pairs of moderately long, and one pair of short setae towards the tip of the small opisthosomal lobes.

Venter

A pair of copulatory suckers with an associated pair of short setae occur near the anus, and the reproductive apparatus is situated in the central metapodosomal region with another pair of minute setae. The perianal setae and one pair of metapodosomal setae which occur on the deutonymph are absent on the adult. The small opisthosomal lobe, however, has one pair of short setae ventrally beyond those noted dorsally.

Legs

Leg I 153–193 (180) (13); leg II 155–198 (182) (15); leg III 219–259 (239) (12); and leg IV 113–138 (121) (10). Legs I and II identical with deutonymph

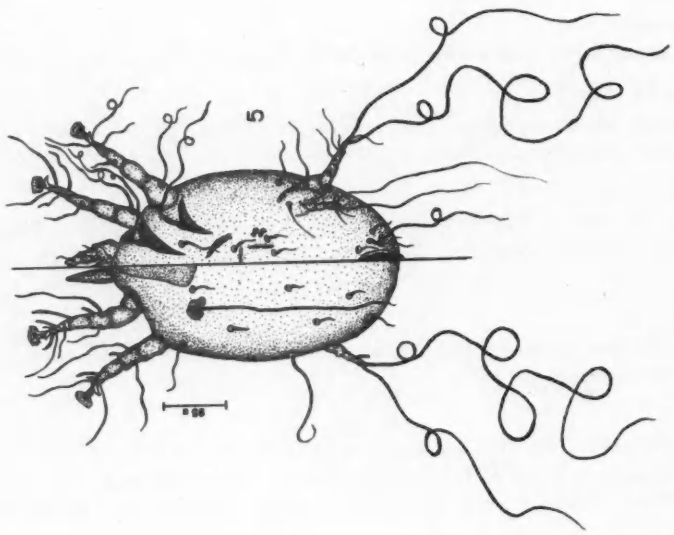


FIG. 5. Dorsoventral view of ovigerous female.

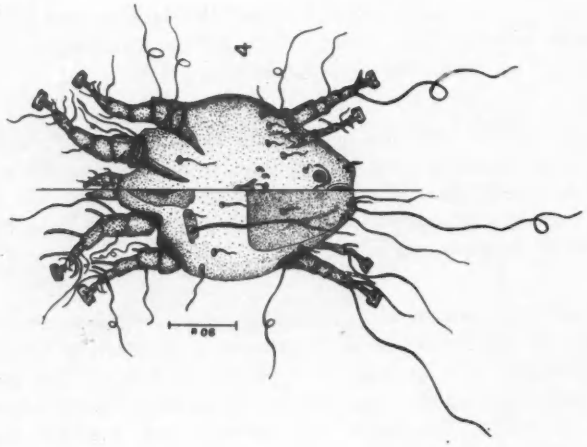


FIG. 4. Dorsoventral view of adult male.

except for size. Leg III has a trochanter with a long seta, tibia with a seta and rod, and tarsus with two quite long setae, a moderately long seta, a biforked hook, and a caruncle. Leg IV has six articles. Tibia IV has a rod and seta, and tarsus IV has one quite long seta, two short setae, a short rod, a hook, and a caruncle.

Gnathosoma

As for other stages. Chelicerae 66–74 (69) (3).

Ovigerous Female (Fig. 5)

Not dissimilar to the immature stages, but leg IV reoccurs, there is a vulva ventrally, and the posterodorsal suckers of the deutonymph do not occur on the ovigerous female.

Dorsum

Length 345–451 (412) (28) and width 270–296 (284) (8). The posterior genital suckers of the deutonymph do not occur on this stage. Setal pattern identical with deutonymph.

Venter

The vulva consists of a transverse slit located in the central podosomal region. Same number of setae as on deutonymph.

Legs

Leg I 171–190 (180) (6); leg II 171–188 (182) (5); leg III 143–164 (154)(7); and leg IV 31–47 (39) (5). Legs I and II identical with deutonymph. Tarsus III has an additional seta beyond those on the deutonymph. Leg IV consists of five articles, with the terminal article supporting two quite long and three moderately short setae.

Gnathosoma

As for previous stages. Chelicerae 69–81 (77) (4).

Egg

The egg is elliptical and white with two bosses towards one end. At the time of larval emergence, the egg splits along the longitudinal axis. Length 166–206 (184) (12).

B. Life History

The life history of *Otodectes cynotis* in some ways resembles, and in other ways differs from, that of its relative *Chorioptes bovis* (15). Under in vitro conditions of 35° C, 80% R.H., and complete darkness, reproducing colonies of *O. cynotis* thrived for about two months on epidermic debris and hair collected from the inside of the pinnae of naturally infested animals. This technique shows that this mite, like *C. bovis*, feeds essentially on dead epidermal material with its chewing chelicerae and that pathology associated with the parasite is caused by something other than direct mechanical injury by the mites.

To observe a stage in the life cycle, eggs or quiescent mites were transferred directly from a domestic cat or a culture vial to an unused vial containing

sterilized epidermic debris and hair from the ears of positive animals. Under the in vitro conditions, the period of incubation of the eggs leading to the emergence of the larva spanned about four days. The larva, and later the protonymph and deutonymph, fed actively and more or less continuously, and gradually increased their size during the motile period. This generally lasted 3 to 5 days, but sometimes persisted for 10 days or even longer on occasion. This proves that the physical environment does not directly control the time span of the mite's motile period. The quiescent period, which followed the motile period, was more regular and varied between 22 and 30 hours. During quiescence, histolysis of the internal tissues occurs, and the tissues within the legs and gnathosoma are resorbed into the body, leaving the legs and body periphery as hollow shells. Immediately preceding ecdysis, it is possible to discern the characters of the next stage. Legs I and II are folded posteriorly over the venter, while legs III and IV and their setae are projected over the venter anteriorly. The old integument is discarded following a transverse slit across the dorsal hysterosoma together with a mediodorsal longitudinal slit through which the new stage emerges.

It will be recalled that the sex of the larva, protonymph, and deutonymph cannot be determined morphologically. The protonymph does not have a pair of suckers at the tip of the opisthosoma, but the deutonymph has, regardless of sex. As in *C. bovis*, soon after the adult male emerges it becomes attached by means of its copulatory anal suckers to the posterior suckers of the deutonymph to form the attachment pair. This attachment is more or less permanent; the male drags the female wherever it goes; and the attachment persists for a variable number of days depending on the state of development of the deutonymph. When completely developed, the adult emerges. Sometimes the emergent adult is an ovigerous female, but, interestingly, part of the time it is another adult male. In some instances, therefore, there occurs an adult male in attachment with a male deutonymph, and the adult male is apparently incapable of determining the sex of the nymph. This peculiarity was verified by mounting attachment pairs with quiescent deutonymphs within which transformation of the adult male was virtually completed. In the case of the close relative *Caparinia ictonyctis*, Lawrence (6) observed two mounted attachment pairs that showed the same oddity.

Experiments followed in the current study to ascertain if there was any biological significance for this peculiarity. Deutonymphs were reared individually. All transformed from the motile into the quiescent state, and an adult male or ovigerous female emerged from the quiescent deutonymph. Those emergent adult males to which deutonymphs were subsequently exposed became attached with these deutonymphs, and those of the latter that transformed into ovigerous females laid eggs. There appears, therefore, to be no physiological significance related to the attachment of adult males with male deutonymphs. The attachment is significant, however, insofar as the female deutonymph is concerned. Ovigerous females resulting from individually raised deutonymphs not exposed to adult males did not lay eggs. (It may be

recalled that the female deutonymphs of *C. bovis* would not enter quiescence, and of course did not molt, except when in attachment with an adult male.) Nor did the ovigerous females of *O. cynotis* lay eggs after adult males were introduced into their vials once the deutonymphal skin was shed. Attachment with the adult male is therefore obligatory if the ovigerous female is to oviposit. Moreover, observations showed that when normally attached pairs were allowed to remain so until after quiescence of the deutonymph was in progress, and then the adult male was pinched off, those deutonymphs that became ovigerous females did not lay eggs. These observations demonstrate that in the case of *O. cynotis*, like *C. bovis*, copulation takes place between the adult male and ovigerous female at a precise moment during the final molt of the female. It shows also that eggs are not laid parthenogenetically.

Females, that had been allowed normal attachment with males, laid their eggs singly. The secretion passed from the vulva at the time of laying solidified upon exposure to the air and firmly attached the egg to the substrate. The complete egg to egg cycle, under the in vitro conditions, ranged widely from 18 to 28 days, or was approximately three weeks.

II. Non-Specificity of Otodectic Mange Mites

A series of names have been applied to the three currently recognized kinds of canker mites. These will be summarized, and then following the description of some experimental results, synonymy will be suggested.

Nomenclature

The otodectic mite was first seen in the domestic dog and named *Sarcoptes cynotis* by Hering in 1838 (*in* Ref. 4). In 1859, Bendz (*in* Refs. 4, 11) recognized the error of including these mites in the genus *Sarcoptes*, and referred them to Gerlach's *Symbiotes*. In the same year, this genus was rejected by Gervais and van Beneden as a homonym and replaced with *Chorioptes* which was adopted for the cat and ferret mites by Mégnin in 1877 (7) and 1878 (*in* Ref. 9) and for the dog mite by Neumann in 1914 (*in* Ref. 9). Sewell (*in* Ref. 8) had adopted *Psoroptes*, but this obvious mistake was not perpetuated. It was Canestrini in 1894 (3) who created the genus *Otodectes* for these mites.

The specific, and sometimes varietal, name has changed as often as the generic name. In 1849, Lucas and Nicolet (*in* Ref. 4) substituted *auricularum* for *cynotis*, and 10 years later Bendz (*in* Ref. 4) used *canis* for the dog mite. Canestrini (3) gave the mites specific rank, but most subsequent authors used *cynotis* in combination with a varietal name. Otodectic mites have been reported (1, 2, 14, 16) from the ranch or red fox (*Vulpes fulva*) and arctic fox (*Alopex lagopus*) and these were referred to the same taxon as the mite from the dog. The naming of the ear mites from the domestic cat and ferret showed a similar historical sequence to that of the dog, and the names and combinations used in previous publications follow in their chronological order according to host. In addition to these, this writer received

skin samples* from the body of a white-tailed deer (*Odocoileus virginianus*) from the Calgary Zoological Garden that contained some *O. cynotis*, and showed marked alopecia over the shoulders, sides, and back. Probably this was an incidental parasite of the deer.

Domestic Dog

Sarcoptes cynotis Hering, 1838.
Sarcoptes auricularum Lucas & Nicolet, 1849.
Symbiotes canis Bendz, 1859.
Psoroptes auricularis var. *canis* Sewell, 1891.
Symbiotes auricularum var. *canis* Neumann, 1892.
Sarcoptes auricularum var. *canis* Railliet, 1893.
Otodectes cynotis Canestrini, 1894.
Chorioptes cynotis var. *canis* Neumann, 1914.
Otodectes cynotis var. *canis* Neveu-Lemaire, 1938.

Domestic Cat

Sarcoptes auricularum var. *cati* Lucas & Nicolet, 1849.
Symbiotes felis Huber, 1860.
Chorioptes ecaudatus Mégnin, 1876.
Chorioptes ecaudatus var. *catotis* Mégnin, 1877.
Psoroptes auricularis var. *canis* Sewell, 1891.
Symbiotes auricularum var. *cati* Neumann, 1892.
Otodectes cynotis Canestrini, 1894.
Chorioptes cynotis var. *felis* Neumann, 1914.
Otodectes cynotis var. *cati* Neveu-Lemaire, 1938.

Ferret

Chorioptes auricularum var. *furonis* Mégnin, 1878.
Symbiotes auricularum var. *furonis* Neumann, 1892.
Otodectes furonis Canestrini, 1894.
Chorioptes cynotis var. *furonis* Neumann, 1914.
Otodectes cynotis var. *furonis* Neveu-Lemaire, 1938.

Comparisons between the Different Mite Varieties

There is little experimental evidence for differentiating the kinds of otodectic mites. In 1892, Railliet and Cadiot (12) were unable to transfer ear mites from dogs to a cat, but were successful in transferring cat mites to a dog and subsequently to still another dog. These same authors were unable to transfer ferret mites to a dog. The current writer obtained live otodectic mites from four sources: domestic dogs, domestic cats, semidomesticated ferrets, and a wild red fox (*Vulpes fulva*). The last host was collected near St. Lazare, Quebec. Mites from each source were compared biologically and morphologically to ascertain if the recognized varieties are truly different.

*Kindly submitted by Dr. H. N. Vance, Veterinary Services Branch, Alberta Department of Agriculture.

The life cycle and habits of the mites from each host were observed in vitro to be identical for each. The period required for a complete life cycle was similar in each case, and varied within the range (18 to 28 days) established previously for mites from the domestic cat. Mites from each source were cultured not only on epidermic debris from their own host but also on epidermal material from each of the other hosts, and in all cases the life cycle was completed in the usual 3-week period. Therefore, the epidermal material from each contained the necessary nutrients for culturing mites from all hosts.

Experiments were conducted to ascertain if mites from different hosts would crossbreed. Any progeny would indicate success since it was shown previously that eggs are not laid parthenogenetically. Quiescent protonymphs from either dogs, cats, or ferrets were raised individually through to deutonymphs, and then males from a different host were introduced. Attachment pairs resulted, while those that persisted as unattached mites were discarded. Those deutonymphs that transformed into ovigerous females laid eggs. Mites from the different hosts, therefore, were compatible reproductively.

Any differences between the types of otodectic mites might be detected when attempting to culture them on epidermal material from the ear of a carnivore not known to be a natural host. The natural hosts occur in three families in the Order Carnivora: Canidae, Felidae, and Mustelidae; so epidermal material from other members of these same families was used in the comparative trials. About 200 eggs from either the dog, cat, or ferret culture were transferred to each of a series of culture vials containing epidermal material from the ears of a red fox, coyote, Canada lynx, fisher, marten, otter, mink, or short-tailed weasel. Table I shows that under the in vitro conditions the results were similar regardless of the original source of the eggs. No differences between mites from the different hosts were, therefore, detectable using this approach.

TABLE I

COMPARATIVE SURVIVAL OF MITES ON EPIDERMAL MATERIAL FROM THE EARS OF RELATED CARNIVORES

Debris source	Egg source		
	Dog	Cat	Ferret
Red fox	C*	C	C
Coyote	–	C	C
Canada lynx	–	D	D
Fisher	D	D	D
Marten	–	P	P
Otter	–	P	P
Mink	P	D	P
Short-tailed weasel	D	P	P

*C: Life cycle completed in the usual 3-week period.
P: Prolongation of the early developmental stages with only the occasional, if any, mite reaching maturity.
D: Death from apparent starvation in the larval stage following the hatching of the eggs.

TABLE II

IN VITRO SURVIVAL OF MITES EMERGENT FROM EGGS ON EPIDERMAL MATERIAL FROM THE EARS OF VARIOUS CARNIVORA

Animal	Individual No.	Period, days: Eggs and larvae	Nymphs†	Adult male	Attachment pair	Ovigerous female	Whole cycle	Summary
Order Carnivora								
Family Ursidae								
Black bear, *Ursus americanus*	1	1–10	7–25	22	23	25	28	C
	2	1–4	N.P.					D
Family Procyonidae								
Raccoon, *Procyon lotor*	1	1–8	7–20	14–18	N.P.*			N
	2	1–8	7–15	15–20	N.P.			N
	3	1–11	N.P.					P
	4 and 5	1–9	N.P.					P
	6	1–8	N.P.					D
Family Mustelidae								
Subfamily Mustelinae								
Fisher, *Martes pennanti*	1	1–9	N.P.					N
Marten, *Martes americana*	1 and 2	1–16	8–24	N.P.				P
Mink, *Mustela vison*	1 and 2	1–10	N.P.					P
Short-tailed weasel, *Mustela cicognanii*	1	1–4	N.P.					D
	2 and 3	1–9	N.P.					N
Ferret, *Putorius putorius*	1	1–11	7–24	17	17	20	24	C
	2	1–8	N.P.					N
Subfamily Lutrinae								
Otter, *Lutra canadensis*	1	1–12	8–24	19–36	N.P.			P
Subfamily Melinae								
Badger, *Taxidea taxus*	1	1–9	7–17	17	N.P.			N
Family Canidae								
Jackal, *Canis aureus*	1	1–4	N.P.					D
	2	1–6	6–30	15–21	N.P.			P
Coyote, *Canis latrans*	1	1–9	5	22	22	24	27	C
	2	1–8	7–29	20–36	N.P.	27	N.P.	P
	3	1–13	7–30	26	N.P.			P
	4	1–8	8–12	N.P.				N
Timber wolf, *Canis lupus*	1	1–8	7–27	15	15	19	22	C
	2	1–7	4–17	N.P.				N
	3	1–9	N.P.					N
Family Felidae								
Canada lynx, *Lynx canadensis*	1	1–4	N.P.					D

*N.P.: Not produced. When N.P. occurs under "Whole cycle", it denotes that all active stages developed, but that the ovigerous females failed to produce eggs.
C: Life cycle completed in the usual 3-week period.
P: Prolongation of the early developmental stages with only the occasional, if any, mite reaching maturity.
N: Cycle progressed on time, but was never completed.
D: Death from apparent starvation in the larval stage following the hatching of the eggs.
†May include one or more nymphal stages.

In the current study, mites from the ears of a cat were transferred to a dog. At autopsy* 3 months later the dog was found infested, and its litter mate, which served as a control, remained negative. This substantiates the previous observation of Railliet and Cadiot (12) that cat mites are capable of reproduction in the ears of dogs. The previous authors reported also that the reverse transfer and a ferret-to-dog transfer were unsuccessful. This, however, may reflect differences in individual, rather than species, susceptibility similar to that demonstrated for *Chorioptes bovis* (15). In the case of otodectic mites, only seven of the nine ferrets observed in this study that were reared in the same pen for many months were infested naturally. Table II shows that in vitro studies carried out in duplicate using about 200 eggs per vial with epidermic debris and hair from inside the ear of one timber wolf, and also from one coyote, were suitable for completion of the mite's life cycle, but epidermal material from other individuals in these same species was only partially suitable. The life cycle was completed also on ear debris from an immature black bear, but on material from another bear the larvae emergent from the eggs died from starvation. Mites on material from a number of other flesh-eating mammals showed prolongation of the nymphal stages, and in the case of a jackal, badger, otter, and two of six raccoons an adult male developed, but the female cycle stopped prematurely. These results show that otodectic mites can survive in vitro to at least some extent on epidermal material from various Carnivora, and there appears to be wide variation in potential susceptibility between individuals in various species.

On the basis of the foregoing data, mites from dog, fox, cat, and ferret are considered biologically indistinguishable. No one has ever stated that the mites differ morphologically, and this writer confirms that those used in this study from the dog, fox, and ferret are identical with those described herein from the cat. These facts demonstrate that perpetuation of the varieties of otodectic mites is untenable, and that all are referable to *Otodectes cynotis* (Hering, 1838) Canestrini, 1894.

Summary

O. cynotis has been reared in vitro for some weeks at 35° C, 80% R.H., and in complete darkness on epidermic debris and hair from inside the ears of carnivores. The life cycle of the mite includes the egg, larva, protonymph, deutonymph, and adult and encompasses about three weeks. The adult male and ovigerous female are morphologically distinct, but all immature stages are indistinguishable. The fourth leg, which is inconspicuous on the protonymph, does not occur on the deutonymph. The adult male forms an attachment pair with the deutonymph which may be of either sex, and when it is a male, there is no physiological significance related to the attachment. Mites from the ears of the dog, wild red fox, cat, and ferret have been shown to be biologically and morphologically identical, and the varietal names of *canis*, *cati*, and *furonis* are declared invalid, and all infestations are referred to *O. cynotis*.

*Observations showed that skinning out the head around the ear canal was the only sure way to ascertain if an animal was infested.

The in vitro life cycle has been completed on ear debris from a coyote, timber wolf, and black bear, and other Carnivora appear to be at least partially suitable, and susceptibility is referable to the individual rather than the species to which it belongs. This parasite was collected also from the body of a captive white-tailed deer.

Acknowledgments

The willing co-operation of Dr. P. J. G. Plummer and Dr. J. W. Pullin, Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, is appreciated. Laboratory assistance was provided by Messrs. D. V. Conlon and J. Mackie. Infested cats were made available by the Department of Physiology, McGill University, and infested ferrets were located in the Department of Pharmacology in the same university. Procurement of epidermal material from the ears of wild carnivores or from raw hides was made possible through the courtesy of Dr. Audrey Fyvie, Research Division, Ontario Department of Lands and Forests; Dr. R. S. Freeman, Department of Parasitology, Ontario Research Foundation; and by officials of the Hudson Bay Co. Mr. R. Standfield, Ontario Department of Lands and Forests, determined the species of the timber wolves and coyotes used in this study.

References

1. ALLEN, J. A. Auricular scabies (parasitic otitis) in silver black foxes. Can. Vet. Rec. **5**, 12–16 (1924).
2. ALLEN, J. A. and WICKWARE, A. B. A preliminary note on parasites infesting domesticated silver black foxes in Canada. Parasitology, **14**, 27–28 (1922).
3. CANESTRINI, G. Prospetto dell'acarofauna Italiana. Part VI*a*. 1894.
4. CANESTRINI, G. and KRAMER, P. Demodicidae and Sarcoptidae. *In* Das Tierreich, Lief. 7. Verlag von R. Friedländer und Sohn, Berlin. 1899.
5. KOUTZ, F. R. Life cycle, diagnosis and treatment of various mites that attack small animals. Vet. Med. **50**, 278–281 (1955).
6. LAWRENCE, R. F. A new mange-mite from the Cape polecat. Ann. Trop. Med. Parasitol. **49**, 54–62 (1955).
7. MÉGNIN, J. P. Monographie de la tribu des sarcoptides psoriques qui comprend tous les acariens de la gale de l'homme et des animaux. Deuxième Partie. Histoire naturelle des acariens qui constituent la tribu des sarcoptides psoriques. Rev. et Mag. Zool. Pur. et Appl. **40**, 82–173 (1877).
8. NEUMANN, L. G. A treatise on the parasites and parasitic diseases of the domesticated animals. *Translated by* G. Fleming. William R. Jenkins, New York. 1892.
9. NEVEU-LEMAIRE, M. Traité d'entomologie médicale et vétérinaire. Vigot Frères, Paris. 1938.
10. OTTEN, E. Beitrag zur Otitis externa parasitaria. Die Bekämpfung der Otodectes-Milbe bei Katze und Hund. Tierärztl. Umschau, **7**, 423–425 (1952).
11. RAILLIET, A. Traité de zoologie médicale et agricole. Part I. Asselin et Houzeau, Paris. 1893.
12. RAILLIET, A. and CADIOT, P. J. Observations et expériences sur l'otacariase symbiotique des carnivores. Compt. rend. soc. biol. **9**, 104–108 (1892).
13. SCHULTZ, C. W. Feline auricular mange. Vet. Med. **33**, 34–35 (1938).
14. STILES, C. W. and Baker, C. E. Key-catalogue of parasites reported for Carnivora (cats, dogs, bears, etc.) with their possible public health importance. Nat. Insts. Health Bull. No. 163. United States Government Printing Office, Washington. 1935.
15. SWEATMAN, G. K. Life history, non-specificity, and revision of the genus *Chorioptes*, a parasitic mite of herbivores. Can. J. Zool. **35**, 641–689 (1957).
16. VITZTHUM, H. G. Acarina *in* H. G. Bronns, Klassen und Ordnungen des Tierreichs, 5. Band: Arthropoda, IV Abteilung: Arachnoidea, 5. Buch. Becker & Erler Kom.-Ges., Leipzig. 1943.

A NEW METHOD TO ESTIMATE LEVELS OF INFESTATIONS OF BLACK-FLY LARVAE (DIPTERA : SIMULIIDAE)[1]

L. S. WOLFE[2] AND D. G. PETERSON[3]

Abstract

A new method of estimating the levels of infestation of black-fly larvae in streams is described. Hollow, metal cones, 20 cm high, 10 cm in diameter at the base, and painted white, were placed in infested streams. The cones were held in position with a wire attached to the apexes and fastened to an object in the stream or on the bank. The larvae attached to the cones in preference to stones or vegetation. Counts of those attached gave estimates of the levels of infestation in the streams, the periods when greatest numbers of larvae were moving downstream, and the fluctuations in number of larvae during the summer.

Introduction

In biological and control studies (6, 9) on black flies near Baie Comeau, Quebec, an improved technique for estimating levels of larval infestations in streams was developed. This paper describes the method, which is based on counting the larvae that attach to a suitable surface placed in streams.

Emergence cages, 1 cu. yd in capacity, were used by Ide (5), Davis (2), and officers of the Entomology Division in northern Canada (7) to study the emergence and succession of black flies. The cages were placed on the stream bed and were examined daily and sometimes hourly. Although this method is excellent for obtaining precise information on the emergence and seasonal succession of black-fly species, it is impractical as an assessment method during large-scale control operations. In assessing treatments of the Saskatchewan River with DDT from an aircraft, Arnason *et al.* (1) sampled areas of the river bed along a staked transect across a rapid infested by *Simulium arcticum* Mall. Thirty to fifty rocks were collected along the staked line and the black-fly larvae were washed off and preserved. The larvae were counted in the laboratory for an index of the level of infestation. In 1954, near Baie Comeau, Quebec, Dr. A. W. A. Brown (personal communication), University of Western Ontario, London, Ontario, used a stone-count method to assess the effectiveness of aerial applications of DDT larvicide. Three observers, using hand tallies, counted all the larvae and pupae on rocks at preselected stations for a period of 5 minutes, for an index of the number of larvae. This exhausting and expensive method was of little value when the number of larvae exceeded 500, since the observers were unable to press hand tallies more than 500 times within the 5-minute period.

Pulpwood logs, 4 ft long and painted white, provide good surfaces for larval attachment when anchored to the stream bank at right angles to the stream flow and weighted so that they remain 4 to 6 in. beneath the water

[1]Manuscript received July 9, 1958.
Contribution No. 3829, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Veterinary and Medical Entomology Unit, Ottawa, Ontario.
[3]Veterinary and Medical Entomology Unit, Ottawa; now at Entomology Laboratory, Guelph, Ontario.

surface (9). However, a long time is required to count the larvae, and the logs frequently become fouled with debris coming down the stream or break loose from the anchoring ropes. Further, only certain types of stream are suitable for anchoring logs.

Black-fly larvae readily attach to surfaces over which the water flow is streamline (3, 4, 9); and light-colored smooth surfaces are preferred (3, 9). These observations suggested that a white cone, placed in the stream with the apex directed upstream, might provide a good surface for larval attachment.

Details of the Method

Hollow, galvanized-metal cones 20 cm in height, 10 cm in diameter at the base, 300 sq. cm in area, with an apex angle of 30°, and painted white are appropriate in size, shape, and color. The cones are held in a stream by lengths of wire attached to the apexes and fastened to logs, stones, or low border vegetation. One meter of attachment wire is usually satisfactory.

Preliminary experiments were conducted with square plates, 100 sq. cm in area, painted white, and attached along a pole. The pole was immersed at various depths in an infested stream, with the faces of the plates at various angles to the direction of the current. Fig. 1 shows a typical example of

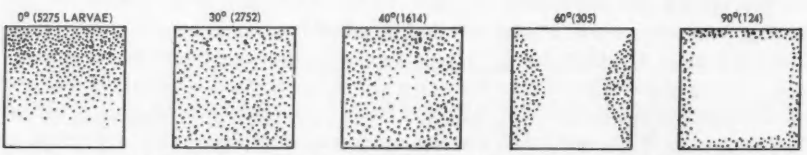


FIG. 1. Distributions and numbers of black-fly larvae that attached to upstream faces of metal plates, 100 sq. cm in area and painted white, placed horizontally and at increasing angles to the flow, in Brisson Creek, Baie Comeau area, Quebec.

the results. The greatest number of larvae attached to plates fixed horizontally to the flow, but the current created areas of turbulance at the upstream edges, preventing the attachment of larvae to the upstream portions. Although fewer larvae attached to the plates set at a 30° angle, the larvae were distributed evenly over the upstream surfaces.

Poles with plates attached were difficult to suspend properly in streams and were not suitable for general use. A small cone of proper dimensions satisfied the requirements for attachment and was more practical. The experiments with the plates indicated that a cone with an apex angle of 60° would be best, provided that it remained suspended in the stream with its axis in the direction of the current flow. Because of its weight, such a cone usually rested with its under surface on the stream bed. The upper surface was then at an angle of approximately 60° to the current flow, resulting in considerable turbulence. The distribution of larvae on the cone was uneven, greater numbers of larvae attaching to the sides and under surface than to the upper surface. A more even distribution was obtained with cones having an apex angle of 30°.

White was selected as the appropriate color as a result of an experiment with black and white cones placed side by side in an infested stream. The larvae attached to each cone were counted every 2 to 4 days from May 26 to July 5. The number of larvae attached on the white cone was always greater than on the black, and usually exceeded it by a factor of 3 to 10.

Selection of cone sites was important. The stream bed was first examined for the presence of larvae, and the cones were then placed in shallow, swift-flowing water. As the season advanced, it was often necessary to move the cones towards the centers of the streams because of the fall in the water level. The density of larvae on the cone was always obviously greater than on surrounding stones or trailing grasses. In many sections of streams, the flow was streamline over only small areas of the stones on the stream bed, and the distribution of larvae on these objects was uneven. When a cone was placed over such stones, larvae attached evenly over its surface. When cones remained clear of larvae at several consecutive inspections and were in a section of the stream with optimum characteristics for larval attachment, extensive examination of the substratum and grasses showed that no larvae were present.

Application of the Method

The advantages of the method are that a simple object is examined on each occasion, only one observer is required, personal error is minimal, and the count is not affected by the rate of counting the larvae found. The method may be used for the following purposes: to estimate the level of infestation at a particular locality; to determine the periods of dispersal of small larvae throughout a stream from oviposition areas; to determine the periods of peak abundance of larvae; and to assess control procedures against the larvae.

TABLE I

BLACK-FLY LARVAE AND PUPAE ATTACHED TO A WHITE CONE* PLACED IN TREMBLAY CREEK, IN BAIE COMEAU AREA, QUEBEC, 1955

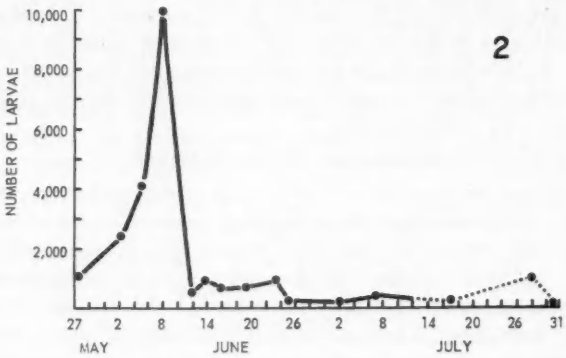
Date	Mean water temperature, °F	Number of larvae		Pupae	Total
		Small†	Large‡		
May 27	50	6	4	0	10
29	48	60	1	1	62
31	47	101	0	2	103
June 2	50	105	112	0	217
3	54	77	11	0	88
6	61	600	272	3	875
8	61	124	1490	20	1634
11	60	66	39	3	108
13	59	78	7	1	86
17	59	296	28	5	329
24	63	766	1384	8	2158
28	69	62	420	4	486
July 1	72	14	56	1	71
5	69	11	170	7	188

*Cleaned after each count.
†First to third instars.
‡Fourth to sixth instars.

A representative sample of a series of cone counts made at various stations throughout the English River drainage basin, near Baie Comeau, Quebec, is shown in Table I. The seasonal changes in the numbers of larvae attaching to cones placed in Tremblay and Brisson creeks, two contrasting types of streams, are shown in Figs. 2 and 3. The interpretation of the data for studies on the dispersal of larvae in a stream, and the development of succeeding generations, is presented elsewhere (9). The method was also employed in experiments on the control of black-fly larvae that will be reported by West (8).

Acknowledgments

We wish to thank Dr. A. S. West, Professor of Zoology, Department of Biology, Queen's University, for his valuable advice and criticism. The



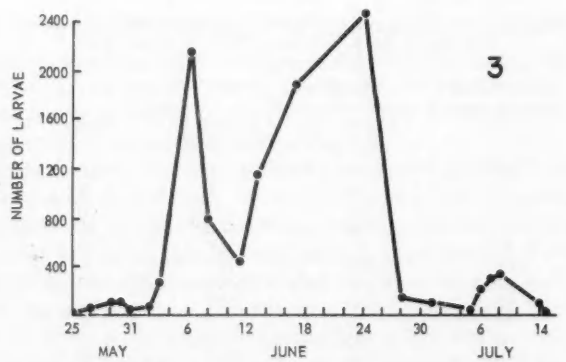


FIG. 2. The seasonal distribution of black-fly larvae, determined by cone counts, in a section of Tremblay Creek in Baie Comeau area, Quebec.

FIG. 3. The seasonal distribution of black-fly larvae, determined by cone counts, in a section of Brisson Creek in Baie Comeau area, Quebec. (The cones were counted at longer intervals after July 12 because of forest fires—Brisson Creek was burnt over on July 28.)

assistance of Mr. B. G. Blair, Veterinary and Medical Entomology Unit, Ottawa, in establishing experimental and assessment stations and in making field observations, is gratefully acknowledged.

References

1. Arnason, A. P., Brown, A. W. A., Fredeen, F. J. H., Hopewell, W. W., and Rempel, J. G. Experiments on the control of *Simulium arcticum* Mall. by means of DDT in the Saskatchewan River. Sci. Agr. **29**, 527–537 (1949).
2. Davies, D. M. A study of the black fly population of a stream in Algonquin Park, Ontario. Trans. Roy. Can. Inst. **28**, 121–159 (1950).
3. Grenier, P. Contribution à l'étude biologique des Simuliides de France. Physiol. Comparata et Oecol. **1**, 165–330 (1949).
4. Hocking, B. and Pickering, L. R. Observations on the bionomics of some northern species of Simuliidae (Diptera). Can. J. Zool. **32**, 99–119 (1954).
5. Ide, F. P. Quantitative determination of the insect fauna of rapid water. Toronto Univ. Studies, Biol. Ser. No. 27; Ontario Fisheries Research Lab. Publ. No. 59 (1940).
6. Peterson, D. G. and Wolfe, L. S. The biology and control of black flies (Diptera : Simuliidae) in Canada. Proc. 10th Intern. Congr. Entomol., Montreal.
7. Twinn, C. R. Studies of the biology and control of biting flies in northern Canada. Arctic, **3**, 14–26 (1950).
8. West, A. S. Control of black-fly larvae in the forests of Quebec by aerial applications of DDT. In preparation.
9. Wolfe, L. S. and Peterson, D. G. Black flies (Diptera : Simuliidae) of the forests of Quebec. In preparation.

STUDIES ON MYTILUS EDULIS L. OF THE "CALANUS" EXPEDITIONS TO HUDSON BAY AND UNGAVA BAY

"CALANUS" SERIES No. 16[1]

I. LUBINSKY

Abstract

The "Calanus" expeditions of the Fisheries Research Board of Canada give the first detailed data on the distribution of *Mytilus edulis* L. in Ungava Bay, Hudson Strait, and the northern part of the Hudson Bay. Types of shells of this mollusc and their growth in the above regions are described. The comparison of the growth of *M. edulis* from Canadian Eastern Arctic, west Greenland, and the northwestern shores of the Atlantic Ocean shows that northward from the Canadian Maritime Provinces the growth of *M. edulis* slows down. In the region investigated by "Calanus", growth diminishes from Ungava Bay to Hudson Bay. The dependence of the distribution of *M. edulis* on the types of water masses and its relation to the northern limits of the subarctic zone in the Canadian Eastern Arctic is discussed.

Our knowledge of the distribution of *Mytilus edulis* L.—the edible mussel—in the Canadian Eastern Arctic has hitherto been very limited. This species has previously been recorded from only two localities in Ungava Bay, namely the mouth of Koksoak River (13) and Cape Hopes Advance (59). It has also been reported from an unspecified locality in Hudson Bay (66), from Fullerton, Roes Wellcome (15), Southampton Island (4), and Chesterfield Inlet (39). It was found in Ashe Inlet in the Upper Savage Island in Hudson Strait (66).

The "Calanus" expeditions of the Fisheries Research Board of Canada, 1947–1953, have shown that this mollusc is widely distributed in the region from Port Burwell on the northeastern shore of Ungava Bay to Churchill on the western shore of Hudson Bay. Specimens of *M. edulis* were taken in Ungava Bay, stations 212, 208, 8, 5, 31, 107, 224, 66, 67, and 215; in Wakeham Bay at station 217; in Hudson Bay at stations 525, 528, 504, 555, 513, and 519. The description of the above stations and their location is given by Dunbar and Grainger (22), Grainger (31), and Grainger and Dunbar (32).

Mytilus was collected exclusively in the intertidal zone. Samples contained 5–15 specimens, with the exception of those from Burwell, where 38 specimens were collected. Numerous empty shells of this mollusc were often found scattered on the shores. Sometimes it was difficult to decide whether they originated from contemporary nearby beds or were washed out from raised ancient beaches, which contain numerous shells of this mollusc (39, 49, 52, 63). It is hard to say whether at present *Mytilus* is as abundant as it has been previously. The "Calanus" expeditions have shown it to be abundant only in a few localities: near Port Burwell, along southwestern shores of Ungava Bay, and in Harbour of Nuvuk.

This paper is based on a limited material and has to be regarded as a preliminary report.

[1]Manuscript received April 18, 1958.
Based on material collected by the "Calanus" expeditions of the Fisheries Research Board of Canada.

TABLE I

DIMENSIONS (IN MM) AND AGE OF SHELLS OF *Mytilus edulis* L. IN HUDSON BAY AND UNGAVA BAY

	Age	L	H	W	H/L	W/L	W/H
				Hudson Bay			
Port Churchill	14	47	25	10	0.53	0.44	0.76
Station 555 L[1]	9	37	18	13	0.48	0.35	0.72
	11	40	18	13	0.45	0.32	0.72
	9	38	18	14	0.47	0.37	0.78
	4	27	14	12	0.51	0.37	0.71
	2	21	11	7	0.50	0.33	0.70
Nuvuk Harbour	12	53	29	25	0.54	0.47	0.89
Station 528 L[6]	11	48	25	21	0.52	0.43	0.89
Station 525 L[5]	8	43	22	20	0.51	0.44	0.98
	10	59	81	25	0.52	0.42	0.80
	10	51	24	20	0.47	0.39	0.85
	5	33	17	15	0.51	0.45	0.88
	0	49	25	22	0.51	0.45	0.88
	4	32	19	14	0.50	0.43	0.79
	4	31	18	15	0.58	0.48	0.83
	3	20	11	10	0.54	0.41	0.90
	3	21	12	9	0.57	0.42	0.75
Wakeham Bay	11	68	35	27	0.57	0.39	0.77
Station 217 L[11]	10	62	29	27	0.46	0.39	0.93
	10	60	25	26	0.41	0.43	1.04
	7	56	27	24	0.47	0.44	0.93
	8	55	31	23	0.56	0.43	0.76
	7	55	29	22	0.53	0.40	0.76
	7	50	24	23	0.48	0.46	0.95
	8	51	26	25	0.50	0.49	0.56
	5	49	24	21	0.50	0.74	0.80
				Ungava Bay			
Southern shores	11	81	38	36	0.47	0.44	0.94
Station 8 L[11]	8	76	37	32	0.49	0.42	0.86
Station 31 L[14]	10	74	34	31	0.46	0.40	0.31
Station 5 L[8]	8	64	31	30	0.49	0.46	0.36
	7	67	33	28	0.49	0.41	0.96
Port Burwell	12	72	36	29	0.50	0.40	0.80
Station 67 L[4]	10	59	32	24	0.54	0.40	0.79
Station 224 L[15]	10	62	31	24	0.50	0.40	0.80
Station 66 L[3]	11	65	39	25	0.60	0.40	0.76
	8	57	31	22	0.54	0.40	0.70
	9	56	30	23	0.53	0.43	0.78
	8	55	26	21	0.50	0.40	0.78
	4	45	23	19	0.51	0.41	0.83
	6	43	22	20	0.51	0.46	0.90
	5	38	19	18	0.50	0.77	0.50
	4	32	16	14	0.50	0.43	0.87
Cape Hopes Advance	7	50	27	22	0.54	0.44	0.82
Station 212 L[8]	7	36	18	18	0.50	0.50	1.07
Station 208 L[7]	3	29	15	12	0.51	0.71	0.80
	5	33	15	14	0.57	0.50	0.74
	6	48	23	23	0.49	0.49	1.00
	7	32	18	13	0.56	0.40	0.89
	3	29	16	14	0.55	0.48	0.88
	5	23	18	11	0.56	0.48	0.84
	6	55	28	22	0.50	0.40	0.79
	5	49	24	18	0.50	0.39	0.73
	4	44	27	22	0.57	0.47	0.81
	5	46	26	20	0.56	0.43	0.80
	4	42	18	17	0.55	0.40	0.80

Shell Types of *Mytilus edulis*

Mytilus in our material varies in size, shape, and color from station to station and to some degree within the same station (Table I). Despite this they form three natural groups: *Mytilus* from the southwestern shores of Ungava Bay, those from Hudson Strait, and those from Hudson Bay. The difference in the length of shells in these groups is considerable (Fig. 1). The individuals of the first group are the largest and those of the third group the smallest. The second group is intermediate and the shells comprising this group vary in size but slightly. *Mytilus* from Burwell can be regarded as representative of this group.

Table II summarizes the data on the length of 5-year-old specimens of these groups. The relations of differences of means to the corresponding errors show that the differences in length in these three groups are significant. Specimens of above groups differ also in the shape of their shells. This shape can be characterized by the ratio of height to length (H/L), width to length (W/L), and width to height (W/H). The mean values of indices of shells of the three groups are shown in Table III. The shells of the first group are low and wide (W/H = 0.89), those of the third group narrow and high.

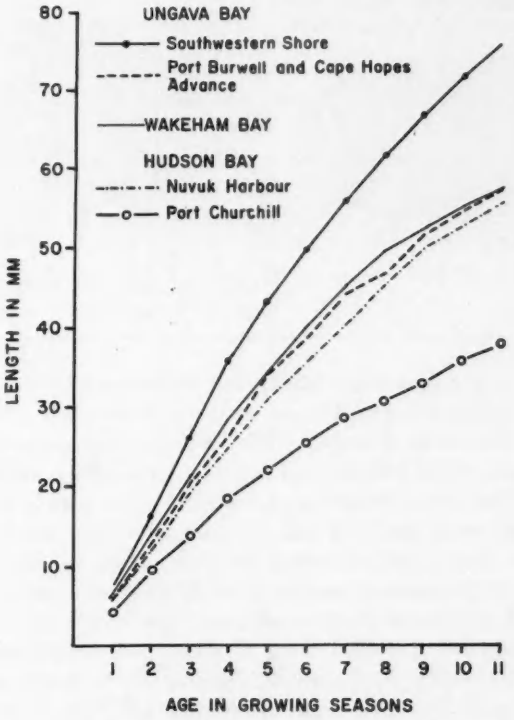


FIG. 1. Growth in length of *Mytilus edulis* L.

The difference of mean values of indices W/H between the second and the third group is insignificant, probably because of the small number of shells from the latter locality. But the difference of mean values of this index of the first group (0.89 ± 0.02; $\sigma = \pm 0.05$) and of the third group (0.75 ± 0.02; $\sigma = \pm 0.05$) is probably significant: $(M_1 - M_2)/\sqrt{\sigma_{M_1}^2 + \sigma_{M_2}^2} = 3.5$. To be significant, for $n = 11$, this relation has to be $\geqslant 3 + 6/(n - 4)$, thus $\geqslant 3.86$.

TABLE II

MEAN LENGTH (IN MM) OF 5-YEAR *Mytilus* FROM DIFFERENT LOCALITIES

Groups	Number of shells	$M \pm \sigma_M$	σ	$(M_1 - M_2)/\sqrt{\sigma_{M_1}^2 + \sigma_{M_2}^2}$
1. Southwestern shores of Ungava Bay	5	41.6 ± 1.23	± 2.70	1–2 gr: 6.1($\geqslant 3.26$)
2. Burwell	22	32.9 ± 0.72	± 3.03	2–3 gr:12.9($\geqslant 3.26$)
3. Churchill	5	22.2 ± 0.53	± 1.18	1–3 gr:21.8($\geqslant 4.0$)

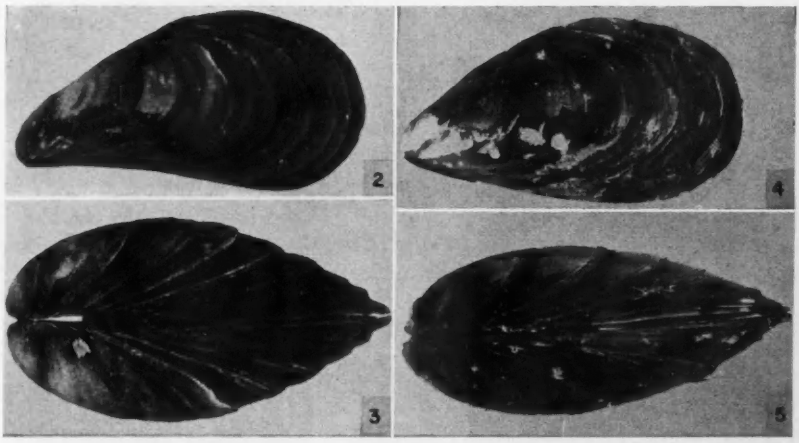
TABLE III

MEAN VALUES OF INDICES H/L, W/L, W/H, OF *M. edulis* FROM DIFFERENT LOCALITIES

Groups	H/L	W/L	W/H	Number of shells
	Mean values of indices			
1. Southwestern Ungava	0.48	0.43	0.89	5
2. Wakeham Bay	0.49	0.43	0.88	9
Cape Hopes Advance	0.53	0.44	0.83	14
Burwell	0.52	0.43	0.83	22
Nuvuk	0.52	0.44	0.85	11
3. Churchill	0.49	0.37	0.75	6

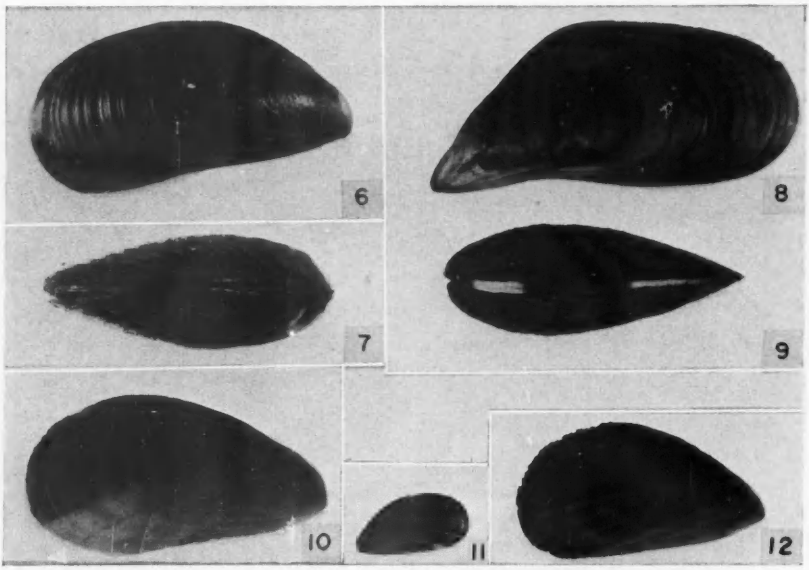
Beside size and proportions, *Mytilus* of these groups differ also in their habitus. Shells from Ungava Bay are heavy, of blue-brown color, their outer surface rough and often damaged. They are flattened dorsoventrally, with very broad base. The anterodorsal ridge and the upper angle are smooth (Figs. 2, 3). The anterior part of the shell is often bent downward. The largest specimen is 89 mm long and 11 years old. The shells of the second group are also heavy, the periostracum dark brown to light gray brown, often lustrous. The marks of arrested growth are clearly visible as dark lines (Figs. 4, 5). The shape of shells is variable. The largest specimen is 72 mm long and 12 years old. *Mytilus* from Churchill are small, black, lustrous. The marks of arrested growth are visible as step-like sculpture on the surface of the shell (Figs. 6, 7). The shells are narrow and their base in transverse section is V-shaped.

PLATE I



FIGS. 2–5 *Mytilus edulis* L. Figs. 2, 3. From southern shores of Ungava Bay, length 8 cm. Figs. 4, 5. From Burwell, length 7 cm.

PLATE II



FIGS. 6–9. *Mytilus edulis* L. Figs. 6, 7. From Churchill, Hudson Bay, length 4.2 cm. Figs. 8, 9. From Liverpool Bay, mouth of Mackenzie River, length 7 cm. Figs. 10–12. *Mytilus pellucidus* Penn. Fig. 10. From Mansel Island, Hudson Bay, length 6 cm. Fig. 11. From Churchill, length 2 cm. Fig. 12. From Diana Bay, Hudson Strait, length 5 cm.

The peculiarity of the shells of the first and partially of the second group is their diverging beaks. These protrude as two tapering tubercles and may reach a length of 3 mm with a distance between the apices of up to 4 mm. The divergence of beaks is more pronounced in dorsoventrally flattened shells with broad base.

Mytilus with diverging beaks were described over a century ago by Lamarck (38(7), p. 46) as *Mytilus borealis*. This *Mytilus* "habite l'ocean boréale de l'Amérique, côté de New York. Aspect de la moule commune ou commestible, mais beaucoup plus grande. Elle en diffère par ses crochets . . . (diverging beaks-Auct.)." Lamarck's specimen was larger than *Mytilus* from Ungava Bay. Similar shells were later described by Reeve (51) in the legend to the "species 20", depicted in his Plate 6: "*Mytilus borealis*—the northern *Mytilus*. A light ovale, swollen shell of a dark shining olive-black colour, tinged with violet at the umbones, which are somewhat removed from each other. Hab. Newfoundland". Shells from Ungava Bay are in many respects similar to those described by Lamarck and Reeve.

According to these authors this peculiar form of *Mytilus* is a separate species. However, in our material it is connected by intermediate forms to the usual form with beaks in close contact. Its peculiar shape can be interpreted as an adaptation to habitats exposed to the mechanical action of ice and currents. Flattening of the shell lessens its lateral surfaces, whereas the widening of its base secures a better attachment. The beaks diverge as a result of extreme widening and bending of the shell. This form is found in the Ungava Bay, where the tidal currents are very strong. The high environmental plasticity of *Mytilus* is well known. It is interesting that some forms of *M. galloprovincialis* Lam. from the Gulf of Naples (2) are similar to those in Ungava Bay.

The second form of *Mytilus* in the "Calanus" collections is *M. pellucidus* Pennant, 1777, with a beautiful pattern of dark purple rays on the shell (Figs. 10, 11, 12). The rays are clearly visible in thinner shells and in those deprived of periostracum. In some of them the pattern expands over the whole surface but usually is represented by a single narrow ray, visible in transmitted light.

Dodge (17) points out some characters peculiar to *M. pellucidus*, which are so constant that he is "strongly inclined to consider it a good species." The presence of rays, however, in shells from our material is not correlated with morphological characters mentioned by Dodge; for example in our shells the upper angle is smooth and situated far from the beaks. The shells are usually dark colored; the light-colored shells from Wakeham Bay and Port Burwell do not exhibit rays. The scarcity of material does not allow us to make conclusions on the range of variability of *Mytilus* in Hudson Bay and in Ungava Bay. Further research is needed to understand the reactions of this species to the Arctic environments and to "find the correlation between the morphological differences and conditions of habitat" (57).

Growth of *Mytilus edulis*

Observations on the growth of this mollusc in Hudson Bay and in Ungava Bay are based on 67 specimens. The length of shells in successive growing seasons was measured on the basis of lines of arrested winter growth. The method used was that discussed by Mossop (44, pp. 3, 4). The results of measurements are represented in Fig. 1, where the shell length is plotted against the time in growing seasons. The curves of growth are S-shaped (Figs. 1, 13). The differences in the rate of growth in different localities are obvious from the shape of the curves and from their relative positions. *Mytilus* from southern shores of Ungava Bay show the highest growth rate; those from Churchill, the lowest. In other localities growth rates vary but slightly and fall between these two extremes (Fig. 1). The difference in size between the fast-and slow-growing shells (from southwestern Ungava and from Churchill) is about 4 mm at the end of the first growing season; it increases to 23 mm by the 6th season, and is about 35 mm in the 11th growing season.

The relative sizes of the shells in different localities remains, however, almost constant throughout the life of the mollusc. The length of shells from Churchill is about half that of shells from the western Ungava Bay in the 1st as well as in the 11th growing season. The length of shells from Burwell is 0.79 that of shells from the southwestern Ungava in the first growing season, and 0.85 in the 11th season. As a result of differences in the growth rates,

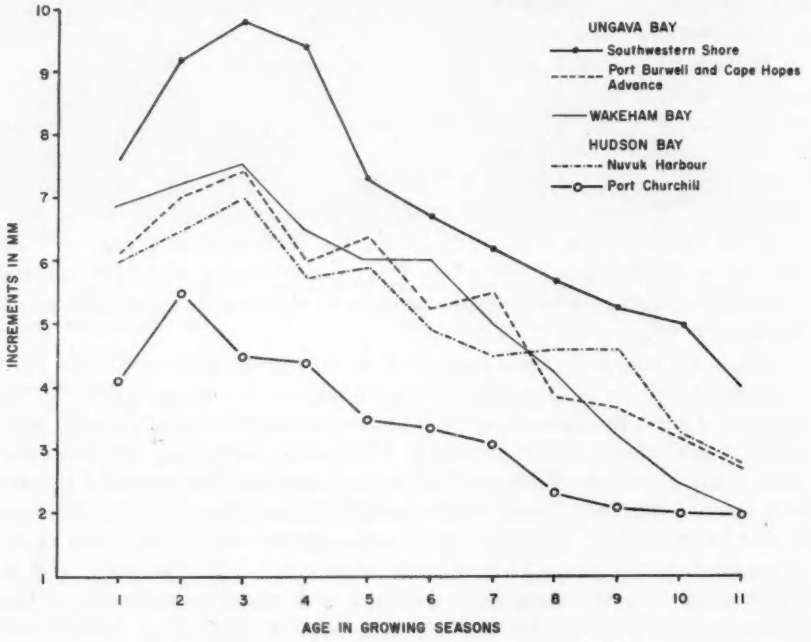


FIG. 13. Annual increments in length of *Mytilus edulis* L.

Mytilus from the southwestern shores of Ungava attain the length of 35 mm in the 4th, from Burwell and Nuvuk Harbour in the 6th, and Churchill in the 10th growing season.

Annual increments as a function of age are represented in Fig 13. The pattern of growth of shells from different localities is almost identical: after a rapid increase in the course of the first three growing seasons with the maximum in the third season, the growth rate gradually decreases. In fast-growing shells the period of rapid growth is prolonged, whereas in slow-growing shells it is limited to the first two growing seasons.

The average annual rate of growth is high in the first two to three growing seasons and decreases with age. In the second five years of life of *Mytilus* it is approximately half of that of the first five years (Tables IV and V).

TABLE IV

THE AVERAGE ANNUAL RATE OF GROWTH OF *M. edulis*

	Age		
	3	5	10
Districts	Average annual rate of growth (in mm)		
Southwestern shores of Ungava Bay	8.8	8.6	7.2
Port Burwell	6.8	6.3	5.5
Churchill	4.6	4.4	3.6

TABLE V

THE AVERAGE ANNUAL INCREMENT DURING THE FIRST AND SECOND 5-YEAR PERIOD OF THE LIFE OF *M. edulis*

	Average annual increment (in mm)	
Districts	First 5 years	Second 5 years
Southwestern Ungava Bay	8.6	4.7
Port Burwell	6.6	3.3
Churchill	4.4	2.4

Discussion

Data on growth of *M. edulis* in the western Atlantic are scarce, and confined mostly to observations on the increment in young mussels (43, 12, 45, 63). In Woods Hole, Mass. (25,54), this mollusc grows to about 75 mm in 5–6 years, in Nova Scotia to the length of 60–80 mm in 4–6 years (43). The shells from Ungava Bay attain this size in about 8–10 years, and in some localities they seldom if ever attain such dimensions (Fig. 1).

The first data on the size of *Mytilus* in consecutive growing seasons were published by Mossop (44). In the first growing season the shells attained the

length of 16 mm in St. Andrews, N.B., and 25 mm in Digby, N.S. Thus they were larger than any shells in our material, which at that age averaged 7–10 mm. The average annual increment of shells in Nova Scotia and New Brunswick varied from 10.8 mm to 16.8 mm, thus was higher than in shells of same age from Ungava Bay (8.6 mm). There is only one note in the literature, that of Mossop (44), on the growth of *Mytilus* in Hudson Bay. According to her the average annual increment of four shells from James Bay, which "appear to be dead when collected", was 8.4 mm, close to that of shells from Ungava Bay.

The distribution and growth of *M. edulis* in the Canadian Arctic is little known. We had the opportunity to examine six specimens of this species collected in the Liverpool Bay near the mouth of the Mackenzie River, now in the collection of the Fisheries Research Board of Canada, Arctic Unit (Table VI). These specimens resemble "species 33*a*" depicted by Reeve (51). The shells are elongated and narrow, with lustrous yellowish-brown periostracum. Valves light, translucent. When deprived of periostracum they are of light blue color with no traces of any pattern. The anterior part of the shell is long, narrow, beaks in close contact. Ridge and angle on the upper margin of the shell are well expressed (Figs. 8, 9). Their growth is fast (Fig. 14). The period of rapid growth extends into the fourth growing season and therefore, from this period on, they are larger than shells of the same age from Ungava Bay. The average annual increment in 6-year-old *Mytilus* from Liverpool Bay is 9 mm, the highest observed in northern latitudes.

TABLE VI

DIMENSIONS (IN MM) OF *M. edulis* FROM LIVERPOOL BAY

L	H	W	H/L	W/L
69.2	28.8	23.5	0.41	0.35
59.0	27.0	19.0	0.46	0.32
58.2	28.5	20.1	0.49	0.34
54.2	25.0	17.5	0.46	0.32
54.2	24.6	21.0	0.45	0.37
53.0	24.0	19.0	0.45	0.36
53.5	25.7	18.8	0.48	0.35
37.8	18.5	13.0	0.49	0.34

On the shores of western Greenland *Mytilus* attains considerable size: the largest specimen collected in Godhavn was 78 mm long; in Upernavik, 72 mm (37). In the Thule district the largest specimen measured 93 mm and was probably over 15 years old (62). Other specimens from the same district were: 4 years old and 44 mm long; 5 years old and 29 mm long. The length of 7-year-old specimens varied from 38 to 59 mm. The annual rate of growth calculated on the basis of these data was 5.4–5.8 mm. and attained a maximum of 8.5 mm. It was thus comparable with the rate of growth of *Mytilus* from Ungava Bay and Hudson Bay.

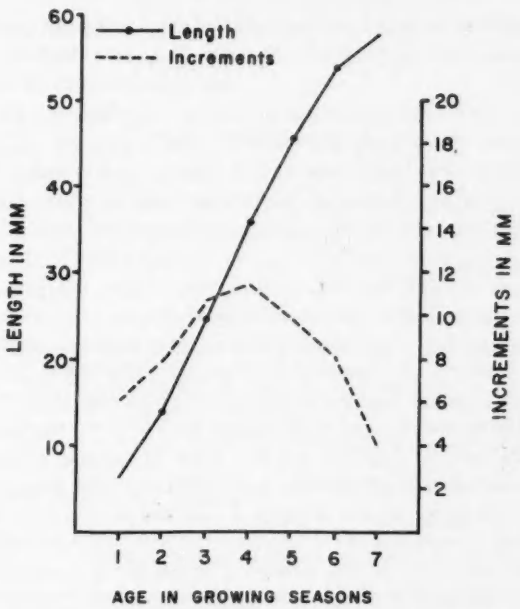


FIG. 14. Growth in length of *Mytilus edulis* L. from Liverpool Bay.

The above data show that north of the Canadian Maritime Provinces the growth of *M. edulis* slows down. This reduction of growth rate in colder waters is a general phenomenon in pelecypods. Thorson (61) pointed out that the growth of arctic species of lamellibranchs is exceedingly slow. The growth of *Mya arenaria* (1, 45, 61), *Mytilus californianus* (10), *M. diegensis*, *Tivela stultorum* (9), *Siliqua patula* (64, 65), as well as of fresh-water bivalves (7), depends on the temperature and on the heat budget of their habitats. It is obvious, moreover, that factors other than temperature influence their growth, e.g. abundance of food, timing of sexual maturity, etc. (48, 61, 8, 9, and many others). Recent observations on the influence of temperature on the activity of poikilotherms show that many species may be relatively independent of this factor. The observations of this type on pelecypods were reported by Spärk (58), Thorson (61), Orton (48), Bruce (5), Fox (26, 28), Rao (50), and their data summarized by Bullock (6). The growth rate, however, does depend on the temperature. "Thus at present there is an apparent contradiction between activity and growth rates" (16, p. 118). As regards gastropods, it has been shown that *Thais emarginata*, *Lacuna carinata*, and *Crepidula nummaria*, while in the egg capsules, grow more rapidly in Alaska than in California (16). Some Cypraeidae and Patellidae are larger in higher latitudes. Other gastropods are larger near the center of their area of distribution, and decrease in size toward the periphery, e.g.

Buccinum undatum, which is often over 100 mm in length near Ireland but attains only half that size in the Barents Sea, near Iceland, and in west Greenland (56).

Weymouth, McMillan and Rich (65) and Weymouth (64) have shown that the northern clams grow more slowly, but live longer and become larger, than the southern ones. The life span of *M. edulis* is not known. The maximum age of specimens in the "Calanus" collections was 14 years; half of the specimens were 10 or more years old. The largest specimen was 8.2 mm long and 11 years old.

Many factors restrict the distribution and inhibit the growth of *Mytilus* in the Arctic: the period of plant and animal growth is short and depends on variable snow and ice conditions (23). Salinity varies considerably and its decrease often affects the growth of *Mytilus*. The inhibition of growth of this mollusc in brackish waters was observed in the Gulf of Bothnia (42), in Kaiser Wilhelm Kanal (3), and in the Baltic Sea (56). In the southern Hudson Bay where the surface salinities are less than $23^0/_{00}$ and mixing of water is poor, *Mytilus* remains small, despite comparatively high temperature. Shells of larger size are found at the mouth of Hudson Bay where salinity attains $32^0/_{00}$, and there is an influx of waters from Foxe Channel and Hudson Strait. In the Ungava Bay, high tides, sweeping across the bay, maintain the circulation, salinity is high and there is an admixture of Atlantic waters (19).

The northern boundaries of the distribution of *M. edulis* in the Eastern Canadian Arctic and in Greenland coincide in general with those of the subarctic zone, as delimited by Madsen (40, 41), and by Dunbar (18, 20, 21). *M. edulis* occurs as far north as Angmagssalik in east Greenland, and Thule in west Greenland. In the Eastern Canadian Arctic it was found, however, near Ponds Inlet (39, 24), thus farther north than the Padloping Island—the northern limit of the subarctic zone in this region (18, Fig. 1). In Hudson Strait it was taken off Southamption Island, and, on the northern shores of Canadian mainland, in the Coronation Gulf. In these last localities the exact position of the boundary of the subarctic zone is not yet known.

Discussing the distribution and growth of *M. edulis* it is necessary to take into consideration the character of water masses in the area. Waters of Canadian Arctic are predominantly of polar character with low salinity and of low temperature. The waters of Hudson Strait, of Ungava Bay, and of west Greenland are of mixed type. The penetration of Atlantic waters can be traced into Ungava Bay, Hudson Strait, and along the shores of west Greenland as far as Thule district (18, 19, 20, 21). As can be inferred from the distribution and growth of *Mytilus*, the best conditions for this mollusc are in the Atlantic waters and to some extent in waters of mixed type. This may explain its better growth in Ungava Bay as compared with Hudson Bay, as well as the penetration of *Mytilus* far northwards along the shores of west Greenland. Its intensive growth in Liverpool Bay may depend on the penetration of Pacific waters, and possibly on the proximity of the estuary of Mackenzie River.

The hydrographic conditions of the Canadian Arctic are complicated, and the latitudinal retardation of the growth of *Mytilus* may be obscured by the peculiarities of its various habitats.

Acknowledgments

I wish to express my gratitude to Dr. M. J. Dunbar, who kindly made available specimens of *Mytilus* from "Calanus" collections, and made many valuable suggestions in the preparation of the manuscript. I am very much indebted to Dr. T. W. M. Cameron for kindly providing facilities for my work.

References

1. Belding, D. A report upon the clam fishery. Mass. 50th Ann. Rept. Comm. Fisheries and Game, Document No. 25. 1916. pp. 93–234.
2. Boetger, C. Die Standortsmodificationen der mediterraneen Miesmuschel *Mytilus* (*Mytilus*) *galloprovincialis* Lam., im Golf von Neapel. Zool. Anz. **91**, 15–23 (1930).
3. Brandt, K. Die Fauna der Ostsee. Verhandl. Deut. Zool. Ges., Leipzig. 1897.
4. Brooks, S. The exploration of Southampton Island. Mem. Carnegie Museum, **12**, (Pt. 2, Sect. 5), 1–2 (1935).
5. Bruce, J. R. The respiratory exchange of the mussel (*Mytilus edulis* L.). Biochem. J. **20**, (1926). Cambridge, Eng.
6. Bullock, J. H. Compensation for temperature in the metabolism and activity of poikilotherms. Biol. Revs. **30** (3), 311–337 (1955).
7. Chamberlain, Th. Annual growth of fresh-water mussels. U. S. Bur. Fisheries Bull. **46**, 713–739 (1931).
8. Coe, W. R. Nutrition and growth of the California Bay mussel, *Mytilus edulis diegensis*. J. Exptl. Zool. **99**, 1–14 (1945).
9. Coe, W. R. Nutrition, growth and sexuality in the Pismo clam (*Tivela stultorum*). J. Exptl. Zool. **104**, 1–24 (1947).
10. Coe, W. R. and Fox, D. L. Biology of the California sea-mussel (*Mytilus californianus*). I. J. Exptl. Zool. **90**, 1–30 (1942).
11. Coe, W. R. and Fox, D. L. Biology of the California sea-mussel (*Mytilus californianus*). III. Biol. Bull. **87**, 59–72 (1944).
12. Coulthard, H. Growth of the sea-mussel *Mytilus edulis* L. Contribs. Can. Biol. and Fisheries, **4**, (N.S.), 121–136 (1929).
13. Dall, W. H. Report on mollusks collected by L. M. Turner at Ungava Bay, North Labrador and from the adjacent Arctic Seas. Proc. U. S. Natl. Museum, **9**, 202–208 (1886).
14. Dall, W. H. Mollusks, recent and Pleistocene. Rept. Can. Arctic Expedition, 1913–18, **8**, (Part A) (Southern Party), 3A–29A (1919).
15. Dall, W. H. Mollusks, recent and Pleistocene. Rept. Can. Arctic Expedition, 1913–18, **8**, (Part A), Suppl. Rept. 31A–35A (1924).
16. Dehnel, P. A. Rates of growth of gastropods as a function of latitude. Physiol. Zool. **28** (2), 115–144 (1955).
17. Dodge, H. A historical review of the mollusks of Linnaeus. Part I. The Classes Loricata and Pelecypoda. Am. Museum Nat. Hist. Bull. 100 (1952).
18. Dunbar, M. J. Note on the delimitation of the Arctic and subarctic zones. Can. Field Nat. **61** (1), 12–14 (1947).
19. Dunbar, M. J. Eastern Arctic waters. J. Fisheries Research Board Can. Bull. 88, (1951*a*).
20. Dunbar, M. J. Resources of Arctic and subarctic seas. Trans. Roy. Soc. Can. **85** (3) (Sect. 5), 61–67 (1951*b*).
21. Dunbar, M. J. Arctic and subarctic, marine ecology: immediate problems. Arctic, **6** (2), 75–90 (1953).
22. Dunbar, M. J. and Grainger, E. H. Station list of "Calanus" Expeditions, 1947–1950. J. Fisheries Research Board Can. **9** (2), 65–82 (1952).
23. Dunbar, M. The pattern of ice distribution in Canadian Arctic seas. Trans. Roy. Soc. Can. **48**, (3rd Ser., Oceanographic Session), 9–18 (1954).
24. Ellis, D. V. Some observations on the shore fauna of Baffin Island. Arctic, **8** (4), 224–236 (1955).
25. Field, I. Biology and economic value of the sea mussel *Mytilus edulis*. Bull. U. S. Bureau Fisheries, **38**, Document 922, 127–259 (1922).
26. Fox, H. M. The activity and metabolism of poikilothermal animals in different latitudes. I. Proc. Zool. Soc. London, **106**, 245–255 (1936).

27. FOX, H. M. The activity and metabolism of poikilothermal animals in different latitudes. III. Proc. Zool. Soc. London, **108**, 915 (1938).
28. FOX, H. M. The activity and metabolism of poikilothermal animals in different latitudes. V. Proc. Zool. Soc. London, **109**, 141–156 (1939).
29. FOX, H. M. and WINGFIELD, C. G. The activity and metabolism of poikilothermal animals in different latitudes. II. Proc. Zool. Soc. London, **107**, 275–282 (1937).
30. FOX, D. L. and COE, W. H. The biology of Californian sea-mussels (*Mytilus californianus*). II. Exptl. Zool. **93**, 205–249 (1943).
31. GRAINGER, E. H. Station list of "Calanus" Expeditions, 1951–52. J. Fisheries Research Board Can. **11** (1), 98–103 (1954).
32. GRAINGER, E. H. and DUNBAR, M. J. Station list of "Calanus" Expeditions, 1953–54. J. Fisheries Research Board Can. **13** (1), 41–45 (1956).
33. GRIEG, J. A. Brachiopods and mollusks with a supplement to the echinoderms. Rept. of the Second Norwegian Arctic Exped. in the "Fram" 1898–1902. Vol. 3, No. 20. 1909.
34. HACHEY, H. B. The general hydrography and hydrodynamics of the waters of Hudson Bay. Contribs. Can. Biol. and Fisheries, **7** (9), 93–118 (1931).
35. HACHEY, H. B. Canadian interest in Arctic oceanography. Artic, **2** (1), (1949).
36. HACHEY, H. B. The hydrography of Hudson Bay. Oceanographic Session. Trans. Roy. Soc. Canada, [3] **48**, 19–25 (1954).
37. JENSEN, AD. S. Lamellibranchiata, 1. The Danish Ingolf Exped. Vol. 2 Copenhagen. 1912.
38. LAMARCK, J. B. Animaux sans vertèbres. Vol. 7. 2nd ed. Paris. 1836.
39. LAURSEN, D. Quaternary shells collected by the Fifth Thule Expedition, 1921–1924 Vol. 1. No. 6. 1937.
40. MADSEN, H. Investigation on the shore fauna of East Greenland with a survey of the shores of other Arctic regions. Medd. Grønland, **100** (8), 1–79 (1936).
41. MADSEN, H. A study of the littoral fauna of Northwest Greenland. Medd. Grønland, **124** (8), 3–24 (1940).
42. MOEBIUS, K. and MAYER, W. Fauna der Kieler Bucht. 1 Bd. Leipzig. 1866.
43. MOSSOP, B. K. A study of the sea-mussel (*Mytilus edulis* Linn.). Contribs. Can. Biol. No. 2, 17–37 (1921).
44. MOSSOP, B. K. The rate of growth of the sea-mussel (*Mytilus edulis* L.) at St. Andrews, New Brunswick, Digby, Nova Scotia, and Hudson Bay. Trans. Roy. Can. Inst. **14** (31), Pt. 1, 3–21 (1922).
45. NEWCOMBE, C. L. Certain environmental factors of a sand beach in the St. Andrews region. J. Ecol. **23**, 233–255 (1935).
46. NEWCOMBE, C. L. Validity of concentric rings of *Mya arenaria* L. for determining age. Nature, **137** (3457), 191–192 (1936*a*).
47. NEWCOMBE, C. L. A comparative study of the abundance and the rate of growth of *Myra arenaria* in the Gulf of St. Lawrence and Bay of Fundy regions. Ecology, **17**, 418–428 (1936*b*).
48. ORTON, J. H. Sea temperature, breeding and distribution of marine animals. J. Marine Biol. Assoc., Plymouth, **12**, (1920).
49. PACKARD, A. S. Observations on the glacial phenomena of Labrador and Maine with a view of the recent invertebrate fauna of Labrador. Mem. Boston Soc. Nat. Hist. **1**, 210–303 (1867).
50. RAO, K. P. Rate of water propulsion in *Mytilus californianus* as a function of latitude. Biol. Bull. **104**, 171–181 (1953).
51. REEVE, L. Conchologia Iconica. Vol. 10. London. 1858.
52. RICHARDS, H. G. Recent and Pleistocene marine shells of James Bay. Am. Midland Naturalist, **17** (2), 528–575 (1936).
53. RICHARDS, H. G. Post-Wisconsin fossils from the west coast of Hudson Bay. Notulae Naturae (Acad. Nat. Sci. Phil.) No. **84** (1941).
54. RICHARDS, O. The growth of the mussel, *Mytilus edulis*, at Woods Hole, Mass. Anat. Record, **64**, Suppl. (1935).
55. SCHILDER, F. A. Einführung in die Biotaxonomie. Jena. 1952.
56. SHIH, C. Y. Die Abhängigkeit der Grösse und Schalendicke mariner Mollusken von der Temperatur u.d. Salzgehalt des Wassers. Sitzungs-Berichte Naturforsch. Freunde. Berlin. 1937.
57. SOOT-RYEN. Studies on the shell proportions of some Norwegian Mytilidae. Nyt Mag. Naturv. **65**, 321–338 (1927).
58. SPÄRCK, R. On the relation between metabolism and temperature in some marine lamellibranchs, and its ecological and zoogeographical significance. Vidensk. Selskabs Biol. Medd. **13** (5) (1936).
59. STIMPSON, W. On the marine shells brought by Mr. Drexler from Hudson Bay. Proc. Acad. Nat. Sci. Phila. **13**, 97 (1861).

60. SWAN, E. F. Growth indices of the clam *Mya arenaria*. Ecology, **33**, 365–374 (1952).
61. THORSON, G. The larval development, growth and metabolism of Arctic marine bottom invertebrates compared with those of other seas. Medd. Grønland, **100** (6) (1936).
62. VIBE, CHR. The marine mammals and the marine fauna of the Thule district. Medd. Grønland, **150** (6), 1–115 (1951).
63. WARREN, E. An ecological study of the sea-mussel. J. Biol. Board Can. **2**, 89–94 (1936).
64. WEYMOUTH, F. The relative growth and mortality in the Pacific razor clam (*Siliqua patula* Dixon), and their bearing on the commercial fishery. Bull. U. S. Bur. Fisheries, **46**, 543–567 (1930).
65. WEYMOUTH, F., MCMILLAN, H., and RICH, W. Latitude and relative growth in the razor clam, *Siliqua patula*. J. Exptl. Biol. **8** (3), 228–249 (1931).
66. WHITEAVES, J. F. Mollusca. Geol. Survey Can., Rept. Prog. 1879–80. Rept. on Hudson's Bay (by Robert Bell). App. 4, 75–76*c* (1881).

ON THE 12-HOOKED ONCOSPHERES OF CANADIAN STRAINS OF ECHINOCOCCUS

G. LUBINSKY[1]

Abstract

Oncospheres of *Echinococcus* possessing 2 to 12 hooks are described. The volume of the 12-hooked oncospheres is almost exactly twice that of normal hexacanths and is surmised that the duodecacanths originate from composite eggs containing two egg-cells.

This paper reports some incidental observations on the variability of the number of hooks in the oncospheres of *Echinococcus*, made in the course of studies on the biology and taxonomy of several strains maintained in this Institute. These studies involved, among other techniques, counting eggs in gravid proglottides cleared with lactic acid. Under these conditions the embryonic hooks were clearly visible and provided adequate material for the study of the variability of their numbers. Over 5000 eggs of the reindeer strain (from Aklavik) and an almost equal number of eggs of the moose strain (from Alberta) were examined. The results of these examinations are summarized in Table I. It will be seen that oncospheres with abnormal numbers of embryonic hooks constituted only 0.23% of the total number of oncospheres examined. The number of hooks varied from 2 to 12, and the *pro mille* of the different variants from 0.09 to 1.0 (Table II). The frequency of occurrence of the variants with a certain number of hooks seems to be proportional, within limits, to this number. Oncospheres with 2 hooks were found only once, those with 12 hooks, 11 times, whereas oncospheres with 4, 5, and 7 hooks were encountered 2, 3, and 8 times respectively.

All the five-hooked oncospheres possessed but one median hook. Six of the seven-hooked embyros had three median hooks (Fig. 2) while two had three lateral hooks. The single two-hooked oncosphere seen was small and obviously degenerate (Fig. 3).

The size of the eleven 12-hooked embryos and of their embryophores was measured and their volume calculated, using the formula for prolate sphaeroides ($\frac{4}{3}\pi ab^2$, where a and b are the major and minor hemiaxes). These figures were compared with those based on measurements of 25 normal embryos from the same population (Table II). As the table shows, the volume of the 12-hooked embryos exceeded that of the normal hexacanths 2.1 times, whereas the volume of their embryophores was only 1.85 times larger than that of the embryophores of normal hexacanths. This may have depended on the fact that the increase in size of the embryophores was not accompanied by a corresponding thickening of their walls.

[1]Manuscript received May 23, 1958.
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.

TABLE I

Strain	Number examined		No. eggs per proglottid	No. oncospheres with:					Total no. of abnormal oncospheres	No. of anomalous oncospheres per 1000
	Proglottids	Eggs		2	4	5	7	12 hooks		
Reindeer	11	5,039	458 (219–734)	–	2	1	6	11	20	3.97
Moose	9	5,810	646 (498–883)	1	–	2	2	–	5	0.89
Total	20	10,849		1	2	3	8	11	25	2.30

TABLE II

RELATIONS OF AVERAGE VOLUMES OF 12- AND 6-HOOKED EMBRYOS OF *Echinococcus granulosus*

	Axes		Volume, μ^3	Relation of volumes
	Long	Short		
12-hooked embryos (11)	42.73 μ	35.8 μ	28,690	2.10
6-hooked embryos (25)	32.60 μ	28.31 μ	13,660	
Embryophores of 12-hooked embryos (11)	57.9 μ	48.98 μ	72,750	1.85
Embryophores of 6-hooked embryos (25)	46.33 μ	40.33 μ	39,410	

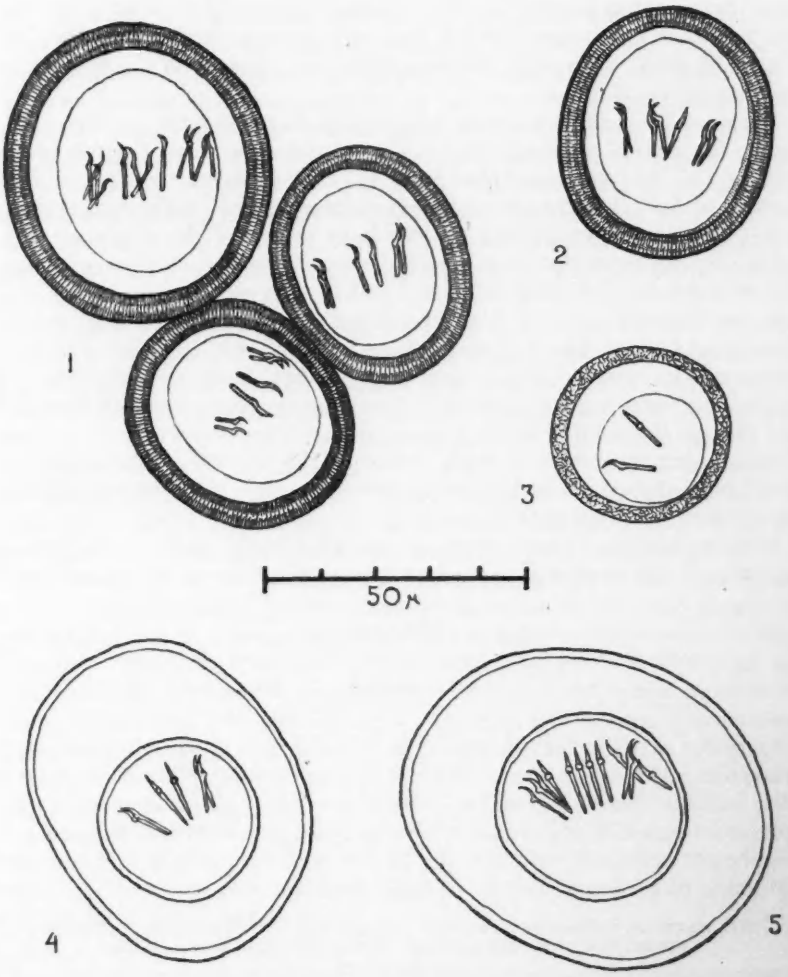


FIG. 1. Hexacanths and 12-hooked oncospheres of *Echinococcus granulosus*. (Experimental infection of a husky from reindeer.) Fig. 2. Seven-hooked oncosphere from the same material. Fig. 3. Two-hooked oncosphere of *E. granulosus*. (Experimental infection of a dog from Alberta moose.) Figs. 4 and 5. Hexacanth and 12-hooked embryo of *Dipylidium caninum* from a dog from Algeria.

Table III shows the relationship between the numbers of hooks and the volume of the oncospheres. It is obvious that there exists a positive correlation between this number and the volume of the embryo (Figs. 1 and 3). The fact that the volumes of the five- and seven-hooked oncospheres are practically equal to those of the hexacanths may depend on the scarcity of the material examined.

The most frequently occurring variant was that possessing 12 hooks, i.e. double the normal number. The duodecacanths were found 11 times in the 5039 eggs of the first population (Table I). The complete absence of oncospheres with 8 to 11 hooks, and of those with more than 12 hooks, was remarkable. Another interesting fact was the large volume of the duodecacanths, almost exactly twice that of the normal oncospheres and of those with five and seven hooks (Table III and Figs. 1 and 2).

It was interesting to check whether these numerical interrelations of the volumes of 12- and 6-hooked oncospheres are valid also for other species of cestodes. On examination of a specimen of *Dipylidium caninum*, collected by Dr. Choquette in Algeria, in two consecutive segments three per thousand and two per thousand of 12-hooked oncospheres were found (Fig. 5). There were no other aberrations in hook number. The relations of the volume of the 12-hooked embryos to that of the hexacanths from the same egg capsules were close to two (Table IV).

It seems that the 12-hooked oncospheres are not the result of the random variation of the number of embryonic hooks, but that of the reduplication

TABLE III

No. hooks	No. oncospheres	Average volume, μ^3
2	1	4,187
4	2	11,070
5	3	13,603
6	25	13,660
7	8	13,316
12	11	28,690

TABLE IV

RELATIONS OF VOLUMES OF 12-HOOKED ONCOSPHERES TO THE AVERAGE VOLUME OF HEXACANTHS FROM THE SAME EGG CAPSULE OF *Dipylidium caninum*

	Length, μ	Width, μ	Volume, μ^3	V12 : V6
12-hooked embryo	39.0	36.0	26,467	1.93
6-hooked embryos (10)	30.0	29.5	13,670	
12-hooked embryo	39.6	36.3	27,467	1.97
6-hooked embryos (10)	30.1	29.7	13,914	
12-hooked embryo	39.0	38.1	29,481	2.14
6-hooked embryos (10)	30.0	29.6	13,768	
Average ratio				2.01

of structures, a process occurring comparatively frequently both in the ontogeny and phylogeny of cestodes. Thus Venard (5), who re-investigated the life cycle of *Dipylidium caninum*, has seen one 12-hooked oncosphere 35 μ in diameter, "in the thousands of oncospheres observed". Yoshina (6) has described 8- to 18-hooked oncospheres of *Taenia solium*, always possessing an even number of hooks. Gaschen (2) found in the hydatid sand from a European pig a scolex with two crowns of hooks. Doubling and trebling of the number of rostellar crowns and of suckers was observed in many species of cestodes, e.g. in *Multiceps serialis* (3). An interesting instance of the doubling of structures is that of *Dioecocestus*, in which the structures are reduplicated only in the male strobilae, but not in the females. The role of the reduplication of structures in the phylogeny of many genera of cestodes was discussed by Baer (1).

The presence of 6 hooks in the oncospheres is an embryonic character peculiar to the class Cestoda in contradistinction to Cestodaria, whose embryo is a 10-hooked lycophore (decacanth). The number of embryonic hooks in cestodes is an extremely constant character, these hooks being absent only in a few genera of the class (e.g. in *Anonchotaenia* and *Cyatocephalus*). An early embryonic trait, characteristic of a class, is not likely to be variable. It is why the terms "oncosphere" and "hexacanth" were always used as synonyms. In the populations of *E. granulosus* examined, 99.77% of the oncospheres were hexacanths, and only about 0.1% duodecacanths.

The frequency of occurrence of the 12-hooked oncospheres in the reindeer strain, 2.2 per thousand, is far in excess of the usual frequency of mutations, and compels one to suspect the involvement of teratogenic factors other than mutations. The large size of the duodecacanths was reminiscent of the experiments of Mangold and Seidel (4), who produced giant embryos of newts by fusion of two dividing eggs. The embryos developing from such eggs possessed single or multiple axial structures depending on the topographic interrelations of the gray crescents of their components. The composite eggs of cestodes usually consist of one egg-cell and of several vitelline cells. It is possible that 12-hooked embryos develop from composite eggs containing two egg-cells, and are thus, so to say, the result of Mangold's experiment made by the oötype of a cestode. Further research is needed to check the correctness of this supposition.

References

1. Baer, J. Ecology of animal parasites. Univ. Illinois Press, Urbana. 1951.
2. Gaschen, H. Anomalie du scolex dans du "sable hydatique". Acta Trop. **5**, 247–248 (1948).
3. Henry, A. Observations sur les anomalies de scolex de *Multiceps serialis* (Gervais). Ann. parasitol. humaine et comparée, **12**, 384 (1934).
4. Mangold, O. and Seidel, F. Homoplastische und heteroplastische Verschmelzung ganzer Tritonkeime. Roux Arch. Entwicklungsmech. **11** (1927).
5. Venard, C. E. Morphology, bionomics and taxonomy of the cestode Dipylidium caninum. Ann. N.Y. Acad. Sci. **37**, 273–328 (1938).
6. Yoshina, K. Studies on the post-embryonic development of *Taenia solium*. J. Formosan Med. Assoc. **32**, 139–141, 166–168 (1933) (in Japanese with English summary).

THE IDENTITY OF THE SPECIES OF LEPEOPHTHEIRUS (COPEPODA) PARASITIC ON PACIFIC SALMON (GENUS ONCORHYNCHUS) AND ATLANTIC SALMON (SALMO SALAR)[1]

L. MARGOLIS

Abstract

A review of existing descriptions of *Lepeophtheirus* from salmonids, based on specimens collected mainly from *Salmo salar* in the European and North American Atlantic and from *Oncorhynchus* spp. in the Asiatic and North American Pacific, coupled with observations by the author on material from *S. salar* from England and from *Oncorhynchus* spp. from a wide range of localities in the North Pacific, suggest that *L. salmonis* (Krøyer, 1838) is the only species found on salmonids from both oceans. The differentiation of *L. uenoi* Yamaguti, 1939 as a distinct species on Pacific salmon seems to be the result of incorrect or inadequate early descriptions of *L. salmonis*.

Lepeophtheirus salmonis (Krøyer, 1838) was originally described from the Atlantic salmon, *Salmo salar*, and is a common external parasite of this salmon in both European and North American Atlantic and adjacent waters. Also it has been recorded many times from five species of Pacific salmon of the genus *Oncorhynchus* in North American and Asiatic Pacific waters. Other anadromous salmonids of the genera *Salmo* and *Salvelinus* also have been reported as hosts. The apparent favorite location of this parasite is in the perianal region of the fish but it may be found on any part of the body or occasionally even on the gills.

Until 1939 the species of *Lepeophtheirus* on Pacific salmonids was considered to be identical with the Atlantic species.[2] Since then Japanese workers have described the species found on *Oncorhynchus* from western Pacific areas as distinct, under the name of *Lepeophtheirus uenoi*. Yamaguti (10) first described this species from specimens collected from *Oncorhynchus gorbuscha* from Hokkaido. In comparing his material with the descriptions of *L. salmonis* as provided by Wilson (9) and Scott and Scott (6) he noted the following differences: (1) "the basal segment of the second antenna has a stout spiniform process on the posterior margin and" (2) "the second segment of the fourth leg is provided at the tip with a well-curved claw" in *L. uenoi*, both of which are lacking in *L. salmonis* according to Wilson (9) and

[1]Manuscript received July 21, 1958.
Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo, British Columbia.

[2]A second species of *Lepeophtheirus*, *L. pacificus* (Gissler, 1883) Wilson, 1905 was described (1, 9) from a salmon (not specifically identified, but probably *Oncorhynchus nerka*) taken in Puget Sound, Washington. Wilson (9) expressed the opinion that on the basis of the original description and illustrations, *L. pacificus* is distinct from *L. salmonis*. Gissler's (1) description lacks many details but nevertheless, contrary to Wilson's opinion, there is a great deal of similarity between Gissler's illustrations of appendages of *L. pacificus* and those of *L. salmonis*. In certain aspects Wilson's illustrations are inaccurate reproductions of Gissler's originals. *L. pacificus* has not been collected again and, according to Wilson (9), the type specimens apparently have been lost. The status of this species must be considered doubtful, but it is not unlikely that it is a synonym of *L. salmonis*.

the Scotts (6). Shiino (7) described *L. uenoi* from *O. keta* taken near the Kurile Islands, and in addition to the differentiating characters cited by Yamaguti, he added the following: "the terminal joint of the exopodite of the 2nd pereiopod bears a simple short spine outside the group of much longer plumose spines in the Atlantic species" (i.e. *L. salmonis*), "while 2 of such are discovered in the Pacific one" (i.e. *L. uenoi*).

Wilson (9), in his description of *L. salmonis*, stated that the basal joint of the second antenna lacks a spine on its posterior margin and that the second joint of the fourth leg has an enlarged tip, with rough papillary elevations, but without a spine. He did not describe the second leg but his illustrations indicate that there is only one small simple spine outside the group of plumose spines on the terminal segment of the exopod of this leg. Scott and Scott (6) wrote that the outer distal angle of the second joint (first joint of the ramus) of the fourth leg is in the form of a blunt knob covered with microscopic bristles. Their illustration of this leg does not show a spine on the second joint. In an earlier paper, the senior author (Scott (5)) remarked that the second joint of the fourth leg "is apparently not provided with a seta in this species, but is simply rounded". His illustration of the fourth leg shows the semicircular flange but no spine on the second segment. The Scotts (5, 6) did not describe or illustrate the basal segment of the second antenna or the second leg.

The collections examined by Wilson (9) came from a variety of sources and from several species of salmonids, including *S. salar* from England and the North American Atlantic coast, and from *Oncorhynchus* spp. caught in Alaska. Although Wilson did not state precisely upon which lot of specimens his description is based, he commented that the specimens from *S. salar* from England were used as the basis for identification of the North American material.

The descriptions by the Scotts (5, 6) were based on British specimens collected from *S. salar* (and possibly *Salmo trutta*).

In redescribing *L. uenoi*, Shiino (7) drew attention to White's (8) statements that specimens of *L. salmonis* taken from *S. salar* of the Canadian Atlantic coast differed from Wilson's (9) and Scott and Scott's (6) description in the possession of a spine on the second joint of the fourth leg. Shiino suggested that White's specimens might actually be *L. uenoi*.

Shiino, as well as Yamaguti (10), apparently neglected descriptions of *L. salmonis* published prior to that of Wilson (9) and also overlooked an important description of *L. salmonis* provided by Gurney (2). Although most of the older descriptions of *L. salmonis* lack many details pertaining to the structure of the appendages, Krøyer (4) had noted the presence of a very small curved thorn or claw on the second joint of the fourth leg (sixth leg of Krøyer) of specimens probably taken from *S. salar* in Denmark. Gurney based his account of the species on material collected from *S. salar* in Great Britain. With reference to the specific characters attributed to *L. uenoi*, he had written of *L. salmonis:* "Wilson states that the basal segment" (of

the second antenna) "lacks the spine on the posterior margin but it is present in my specimens." He further described the terminal segment of the exopod of the second leg "with 5 feathered setae and 3 outer spines, of which the distal one is very large." This distal spine actually bears bristles on one side, as seen in Gurney's illustration, thus leaving only two non-plumose small spines as described for *L. uenoi*. His illustration of this structure is in perfect agreement with those of Yamaguti and Shiino for *L. uenoi*. In spite of Gurney's statement that segment 2 of the fourth leg is without a spine, his figure of this leg (Fig. 1967, p. 333) clearly indicates a small curved spine on the outer distal angle, which is supposed to be characteristic of *L. uenoi*. Consequently Gurney's *L. salmonis* and *L. uenoi* are apparently identical.

Detailed illustrations of a female specimen of *L. salmonis* taken from *Leuciscus brandti* (an unusual host), recently published by Gusev (3), demonstrate the presence of the characteristics of the second antennae and second and fourth pereipods attributed by Japanese workers to *L. uenoi*. Basing his opinion on a re-examination of specimens from *S. salar* from the White Sea and from *O. gorbuscha* from the Okhotsk Sea, contained in the collection of the Zoological Institute of the Academy of Sciences of the U.S.S.R., Gusev concluded that *L. uenoi* was probably a synonym of *L. salmonis*.

Through the courtesy of Mr. R. U. Gooding, formerly of the Marine Laboratory, Plymouth, England, I have been able to examine a collection of *Lepeophtheirus* taken from *S. salar* from the River Tamar, England. None of this material agrees with the concept of *L. salmonis* as published by Wilson (9) and Scott and Scott (6), but all specimens fit Gurney's (2) account or illustrations of *L. salmonis*. I have also had the opportunity to collect and examine numerous specimens of *Lepeophtheirus* from five species of Pacific salmon, particularly *O. nerka* and *O. gorbuscha*, taken from many localities along the North American coast and from waters close to Asia, and from *Salmo clarki* from the British Columbia coast. All of these Pacific collections have been found to be morphologically identical with the specimens from *S. salar* which I have examined.

The descriptions and/or illustrations of Krøyer (4), Gurney (2), Yamaguti (10), White (8), Gusev (3), and Shiino (7), and personal observations of *Lepeophtheirus* from *S. salar* from European and North American Atlantic seas and from *Oncorhynchus* spp. from Asiatic and North American Pacific waters, therefore, suggest that only one species is involved and that its name should be *L. salmonis*, with *L. uenoi* as a synonym. It seems likely that errors or omissions in Wilson's and the Scotts' descriptions of *L. salmonis* have led to the differentiation of *L. uenoi*.

References

1. Gissler, C. F. A new parasitic copepod crustacean. Am. Naturalist, **17**, 885–887 (1883).
2. Gurney, R. British fresh-water Copepoda. Vol. III. Ray Society, London, 1933.
3. Gusev, A. V. Parasitic copepods of some marine fishes. (In Russian.) Parazitol. Sbornik Akad. Nauk S.S.S.R. **13**, 394–463 (1951).

4. KRØYER, H. N. Bidrag til Kundskab om Snyltekrebsene. Naturh. Tidsskr. 3R, **2**, 75–320 (1863).
5. SCOTT, T. Notes on some Crustacean parasites of fishes. 18th Ann. Rept. Fisheries Board Scotland (1899), Pt. III, 144–188 (1900).
6. SCOTT, T. and SCOTT, A. The British parasitic Copepoda. Vol. I and Vol. II. Copepoda parasitic on fishes. Ray Society, London. 1913.
7. SHIINO, S. M. Copepods parasitic on Japanese fishes. I. On the species of *Caligus* and *Lepeophtheirus*. Rept. Faculty Fisheries, Prefectural Univ. Mie, **1**, 79–113 (1952).
8. WHITE, H. C. "Sea Lice" (*Lepeophtheirus*) and death of salmon. J. Fisheries Research Board Can. **5**, 172–175 (1940).
9. WILSON, C. B. North American parasitic copepods belonging to the family Caligidae. Part I. The Caliginae. Proc. U.S. Natl. Museum, **28**, 479–672 (1905).
10. YAMAGUTI, S. Parasitic copepods from fishes of Japan. Part 5. Caligoida, III. Jubilee Vol. Prof. Sadao Yoshida, **2**, 443–487 (1939).

A NEW SPECIES OF LECITHOPHYLLUM FROM NORTH PACIFIC FISHES WITH A CONSIDERATION OF THE TAXONOMY OF THE GENERA LECITHOPHYLLUM, APONURUS, AND BRACHADENA (TREMATODA: HEMIURIDAE)[1]

L. MARGOLIS

Abstract

Lecithophyllum anteroporum n. sp. is described from *Merluccius productus* from British Columbia waters. Other hosts in the North Pacific are *Oncorhynchus nerka* and *O. gorbuscha*. The status of the genera *Lecithophyllum* Odhner, *Aponurus* Looss, and *Brachadena* Linton, and allocation of species within these genera are discussed. Keys to separate the three genera and the four species of *Lecithophyllum* are presented. *Aponurus intermedius* Manter is transferred to *Lecithophyllum* with *L. fuscum* Yamaguti as a synonym.

Eight specimens of an apparently undescribed species of *Lecithophyllum* were collected from the hake, *Merluccius productus* (Ayres), caught off the mouth of the Fraser River, British Columbia, on October 25, 1957. One and three specimens, respectively, were found in the stomachs of two hake and the other four specimens were recovered from the stomach washings of the remaining two fish, which were examined together. Prior to collecting the trematodes from *M. productus* the same species had been taken from adult *Oncorhynchus nerka* (Walbaum) and *Oncorhynchus gorbuscha* (Walbaum), two species of Pacific salmon.

Data on the occurrence of the trematode in *O. nerka* and *O. gorbuscha* are given in Table I. A total of 31 specimens, 25 from *O. nerka* and 6 from *O. gorbuscha*, were encountered, representing infections in 15 of 1389 *O. nerka*, and 2 of 1017 *O. gorbuscha* examined. The *Oncorhynchus* spp. were taken in 1955 and 1956 in the North Pacific area from the North American coast to the Sea of Okhotsk and Hokkaido. The salmon were frozen immediately after capture and subsequently shipped to the Fisheries Research Board, Biological Station, Nanaimo, where they were thawed and examined. The co-operation of the Japanese Fisheries Agency, the United States Fish and Wildlife Service, and various staff members of this Station in providing the salmon samples, as part of the research program of the International North Pacific Fisheries Commission, is gratefully acknowledged.

The low incidence of *Lecithophyllum* in *Oncorhynchus* spp. suggests that these fishes are incidental hosts. *Merluccius productus* is probably a normal definitive host.

Of the specimens collected from *M. productus*, four were killed and fixed by being dropped into hot (70° C) alcohol – formalin – acetic acid solution and four into hot Bouin's solution. Those from *Oncorhynchus* were killed during the freezing of the host and subsequently were fixed in 10% formalin.

[1]Manuscript received July 21, 1958.
Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo B.C.

TABLE I

RECORDS OF COLLECTION OF *Lecithophyllum anteroporum* N. SP. FROM *Oncorhynchus nerka* AND *O. gorbuscha* IN THE NORTH PACIFIC

Locality	Date of capture	No. of fish examined	No. of fish infected	No. of specimens in each fish
Oncorhynchus nerka				
Fraser R., British Columbia	4–8–1955	25	2	1, 10
Rivers Inlet, British Columbia	18–7–1956	25	1	1
Skeena R., British Columbia	21–7–1955	25	2	1, 1
Skeena R., British Columbia	18–7 to 5–8–1956	25	1	1
Seldovia, Cook Inlet, Alaska	21–7–1955	25	1	1
Karluk R., Kodiak Is., Alaska	22–6–1956	25	1	1
51° N. lat., 175° W. long.	8–8–1956	20	1	1
Attu, Aleutian Is., Alaska	4–8–1955	25	1	1
48° 43′ N. lat., 170° 23′ E. long.	18–5–1955	7	1	1
48° 35′ N. lat., 168° 36′ E. long.	1–6–1955	10	1	1
51° 04′ N. lat., 164° 08′ E. long.	25–7–1956	25	1	2
51° 40′ N. lat., 159° 50′ E. long.	5–8–1956	25	1	1
51° 55′ N. lat., 154° 13′ E. long.	6–7–1956	25	1	1
Oncorhynchus gorbuscha				
Fraser R., British Columbia	3–10–1956	16	1	1
Ketchikan, Southeast Alaska	7–8–1956	25	1	5

The trematodes were stained with Gower's alum carmine or Grenacher's borax carmine, cleared in beachwood creosote, and mounted in Canada balsam. One of the specimens from *M. productus* (fixed in Bouin's) and four specimens from *Oncorhynchus* were sectioned sagittally or frontally and stained in Delafield's haematoxylin. Only the Bouin's-fixed specimen gave satisfactory sections.

The following description is based on the specimens from *M. productus* since stained whole mounts, as well as sectional material, were superior to those prepared from specimens collected from *Oncorhynchus*.

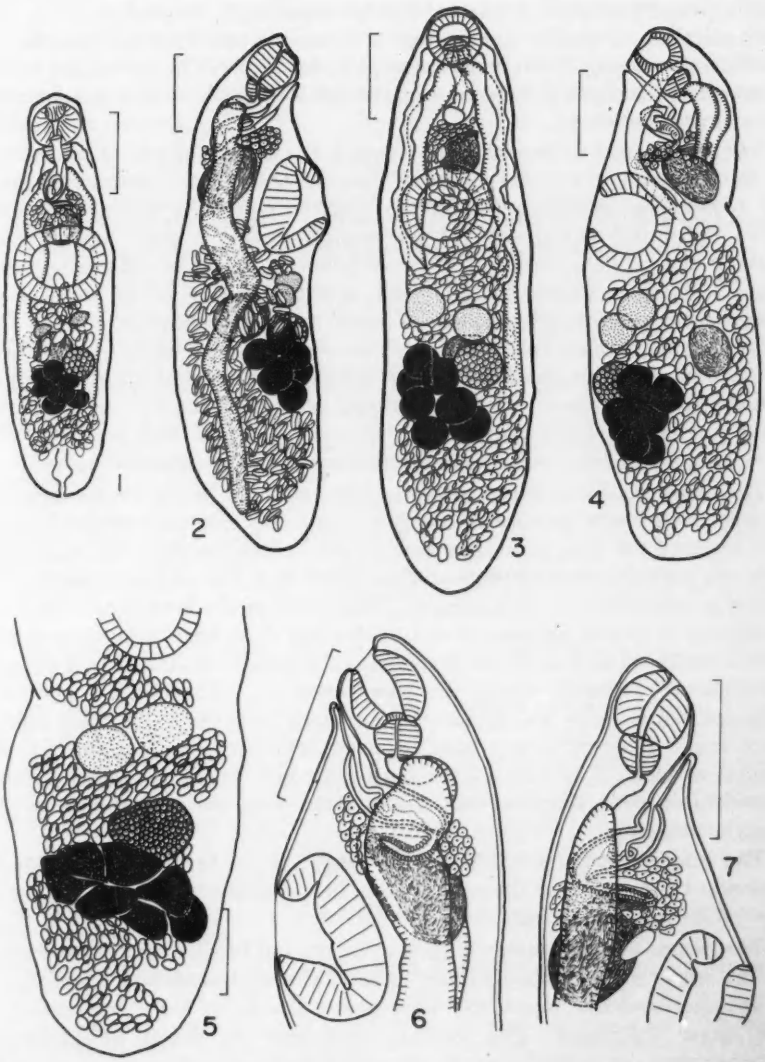
Lecithophyllum anteroporum n. sp.

(Figs. 1–6)

Description

The body is more or less cylindrical with broadly rounded anterior and posterior extremities. In ventral aspect the sides are nearly parallel behind the acetabulum and taper anterior to the acetabulum. Mature specimens measure 0.97 to 1.47 mm in length with a maximum breadth or depth of 0.263 to 0.355 mm. The forebody is about $\frac{1}{4}$ to $\frac{3}{10}$ of the total body length. The length of the subterminal oral sucker is usually close to $\frac{1}{2}$ the length of the acetabulum.

The subglobular pharynx, about $\frac{1}{2}$ of the length of the oral sucker, lies posterodorsal to this sucker. A short esophagus, directed posterodorsally, gives rise to two wide caeca that become narrower posteriorly. They run dorsally and terminate near the posterior extremity of the body, at about the same level or a little in front of the hindmost uterine loops.



FIGS. 1–6. *Lecithophyllum anteroporum* n. sp. FIG. 1. Ventral view of specimen from *Merluccius productus*. FIG. 2. Lateral view of specimen from *M. productus*. FIG. 3. Ventral view of specimen from *Oncorhynchus nerka*. FIG. 4. Lateral view of specimen from *O. nerka*. FIG. 5. Hindbody of specimen from *O. nerka*, compressed before fixation. FIG. 6. Lateral view of forebody of specimen from *M. productus*. FIG. 7. *Lecithophyllum sphaerolecithum* (Manter). Lateral view of forebody. All figures drawn with the aid of a camera lucida. Scale = 0.2 mm.

The diagonally arranged tests are oval to round in outline and lie a little behind the acetabulum near the ventral surface of the body. Although their positions relative to one another are variable, the left testis is anterior and dorsal to the right one more frequently than not. The size of the testes is not related to body size, smaller specimens sometimes having larger testes than larger specimens.

The pear-shaped to ovoidal seminal vesicle lies dorsal and partially anterior to the acetabulum with its long axis directed obliquely dorsoventrally and the broad base extending behind the anterior margin of the acetabulum. From the anteroventral apex of the seminal vesicle a short narrow duct leads to the prostatic duct. The latter follows a dorsal or obliquely dorsal course, sometimes folding back on itself, to enter the base of the hermaphroditic pouch subterminally, where it joins the hermaphroditic duct. The prostatic duct varies in relative length from about $\frac{2}{3}$ to almost equal the length of the hermaphroditic pouch. The prominent prostatic cells are not uniformly distributed along the entire length of the prostatic duct but are arranged mainly in two groups, a dorsal and a ventral. Some of these prostatic cells have long ducts which open into the mid-region of the prostatic duct.

The coiled or folded hermaphroditic duct, enclosed in the hermaphroditic pouch, opens into a prominent genital atrium. Its basal portion is enlarged and receives the pars prostatica and uterus. The portion that protrudes into the genital atrium is of greater diameter than the adjacent portion enclosed in the hermaphroditic pouch. The length of the hermaphroditic duct is difficult to determine because of its folds and coils, but it is at least equal to the combined lengths of the hermaphroditic pouch and the genital atrium, thus exceeding the length of the pars prostatica. The elongate–pyriform hermaphroditic pouch is directed anteroventrally from the end of the prostatic duct and is frequently bent back on itself before reaching the base of the genital atrium. The base of the hermaphroditic pouch is always located considerably more than one-half the distance from the genital pore to the acetabulum.

The genital atrium, about $\frac{1}{3}$ to $\frac{2}{3}$ the length of the hermaphroditic pouch, opens to the exterior, as the genital pore, in a median, transversely oval slit, ventral to the oral sucker.

The ovary, oval to round in outline, is situated behind the testes, a little to the left or right of the mid-line, close to the ventral surface of the body. It is larger than the testes, the difference in size being more pronounced in the larger specimens. The seminal receptacle lies dorsal and partially anterodextral (or anterolaevo) to the ovary and is about equal in size with it except in the smallest and youngest (based on number of eggs in the uterus) specimen in which it is only one-half the diameter of the ovary.

The vitellaria consist of seven lobes, round or oval to pyriform in outline, arranged in two distinct groups of four and three lobes each. The group of four lobes lies to the right or left of the ovary (depending on whether the ovary is to the left or right of the mid-line) and partially posterior to it, and

the other group lies behind these and the ovary. The seven lobes do not unite at a common central point, but in some specimens the lobes within each group were clearly observed to be united. There is variation in the size of individual vitelline lobes in a specimen, but there is no difference between the sizes of lobes in the two groups. All vitelline lobes are smaller than the ovary.

TABLE II

MEASUREMENTS (IN MILLIMETERS, EXCEPT EGGS IN MICRONS) OF MATURE *Lecithophyllum anteroporum* N. SP. FROM *Merluccius productus* AND *Oncorhynchus* SPP.

	M. productus		*Oncorhynchus* spp.	
	Range	Mean*	Range	Mean*
Total length	0.97 –1.47	1.19 (8)	0.91 –1.49	1.23 (23)
Maximum breadth or depth	0.248–0.355	0.293 (8)	0.278–0.477	0.360 (23)
Forebody length	0.291–0.419	0.328 (8)	0.277–0.398	0.334 (23)
Oral sucker				
Length	0.103–0.128	0.111 (8)	0.096–0.148	0.115 (23)
Width or depth	0.101–0.136	0.118 (8)	0.095–0.135	0.116 (23)
Pharynx				
Length	0.047–0.071	0.058 (8)	0.052–0.084	0.065 (23)
Width and depth	0.058–0.085	0.074 (8)	0.051–0.082	0.066 (23)
Esophagus length	0.063–0.077	0.068 (6)	0.054–0.079	0.064 (7)
Acetabulum				
Length	0.182–0.248	0.214 (8)	0.176–0.280	0.218 (23)
Width	0.186–0.254	0.222 (4)	0.176–0.276	0.208 (8)
Depth	0.126–0.189	0.163 (8)	0.155–0.245	0.203 (20)
Ratio oral sucker length to acetabulum length	1:1.67–1:2.10	1:1.92 (8)	1:1.76–1:2.41	1:1.91 (23)
Seminal vesicle				
Length	0.126–0.221	0.167 (8)	0.126–0.245	0.155 (21)
Maximum width	0.087–0.122	0.098 (8)	0.064–0.128	0.095 (23)
Prostatic duct length	0.111–0.150	0.132 (7)	0.089–0.146	0.111 (13)
Hermaphroditic pouch				
Length	0.158–0.186	0.169 (7)	0.114–0.178	0.150 (14)
Maximum width	0.041–0.071	0.057 (8)	0.040–0.073	0.052 (20)
Genital atrium length	0.063–0.122	0.080 (7)	0.082–0.160	0.123 (13)
Testes				
Length	0.030–0.090	0.062 (8)	0.071–0.150	0.100 (20)
Width or depth	0.047–0.095	0.067 (8)	0.076–0.170	0.113 (20)
Ovary				
Length	0.084–0.131	0.104 (8)	0.095–0.176	0.123 (18)
Width or depth	0.079–0.122	0.095 (8)	0.098–0.181	0.131 (18)
Seminal receptacle				
Length	0.085–0.131†	0.107 (7)	0.071–0.142	0.108 (8)
Width or depth	0.088–0.147†	0.109 (7)	0.071–0.119	0.095 (8)
Vitelline lobes				
Length	0.035–0.119	0.071 (8)	0.064–0.126	0.092 (16)
Width or depth	0.038–0.098	0.070 (8)	0.064–0.114	0.090 (16)
Postvitelline body length	0.152–0.369	0.254 (8)	0.184–0.361	0.270 (23)
Ova				
Length	42.7–61.6	49.8 (8)‡	38.0–60.0	45.9 (25)‡
Width	23.7–30.0	25.5 (8)‡	20.5–28.4	25.5 (25)‡
Mean length in individual specimens	44.8–55.8	49.8 (8)‡	40.9–54.6	45.9 (25)‡
Mean width in individual specimens	24.6–25.9	25.5 (8)‡	22.2–27.5	25.5 (25)‡

*Number of specimens upon which the mean was based is shown in parentheses.

†Does not include measurement of smallest specimen in which the seminal receptacle, 0.044 × 0.046 mm, was only about one-half the diameter of the ovary.

‡Ten eggs measured in each specimen.

The uterus, filled with ovoidal, operculate ova, occupies most of the available space in the hindbody. In the forebody a single loop of the uterus passes anteroventrally between the acetabulum and the seminal vesicle. It turns dorsally over the seminal vesicle, closely following the pars prostatica, and enters the base of the hermaphroditic pouch to join the hermaphroditic duct ventrolateral to the pars prostatica. The average egg size based on measurements of 10 eggs in each of eight specimens is 49.8μ by 25.5μ. The complete range in size is 42.7 to 61.6μ in length by 23.7 to 30.0μ in width. Although the variation in length is 18.9μ and in width 6.3μ, the maximum variation in any one specimen is 9.4μ in length and 4.7μ in width. The mean egg size of individual specimens varied from 44.8 to 55.8μ in length and 24.6 to 25.9μ in width.

The excretory pore is terminal. The main excretory duct bifurcates in the testicular region, giving rise to two branches which run anteriorly in the lateral fields, ventral to the caeca, and unite dorsal to the pharynx. A bulbous enlargement of the main duct occurs a short distance anterior to the pore.

Measurements of mature specimens of *L. anteroporum* from *M. productus* and *Oncorhynchus* spp. are given in Table II.

Some differences are noted between the specimens from *Merluccius* and those from *Oncorhynchus*. Specimens from the latter host genus are stouter, have a pharynx that is more nearly spherical, a greater ventrodorsal diameter (depth) of the acetabulum, generally larger gonads and vitellaria, and frequently slightly smaller eggs. The vitelline lobes, although usually smaller than the ovary, as in the specimens from *Merluccius*, occasionally are as large as the ovary. These differences between specimens from the two host genera are not important enough to warrant consideration of the existence of two distinct species. The development in different hosts and/or the effects of the different methods of killing and fixation probably account for the observed dissimilarities.

Type host: Merluccius productus (Ayres), hake (family Merlucciidae).

Other hosts: Oncorhynchus nerka (Walbaum), sockeye salmon; *Oncorhynchus gorbuscha* (Walbaum), pink salmon (family Salmonidae).

Type locality: Mouth of the Fraser River, British Columbia.

Geographical distribution: North Pacific, from British Columbia and Alaska to the Sea of Okhotsk.

Type specimens: Holotype and paratypes to be deposited in the National Museum, Ottawa, Canada.

Discussion

The genus *Lecithophyllum* was created by Odhner (21) to accommodate *Distoma botryophoron* Olsson, 1868 (24). Three other species have since been assigned to this genus: *L. fuscum* Yamaguti, 1938 (31), *L. sphaerolecithum* (Manter, 1925) Manter, 1947 (14, 15, 19), and *L. pyriforme* (Linton, 1910) Yamaguti, 1953 (9, 34).

Manter (19) reduced *L. fuscum* to the synonymy of *Aponurus intermedius* Manter, 1934 (17). Although a genital atrium was not described for *A. intermedius*, as in *L. fuscum*, its presence is indicated in the illustration since the genital pore is shown some distance in front of the hermaphroditic pouch. There have been conflicting concepts of the two genera but the presence of a prominent genital atrium in *Lecithophyllum* and its absence in *Aponurus* appears to be the significant difference between them as will be noted later in this paper. Therefore, *Aponurus intermedius* should be transferred to *Lecithophyllum*, with *L. fuscum* as a synonym.

Lecithophyllum sphaerolecithum was returned to its original generic designation, *Aponurus*, by Yamaguti (34) because a genital atrium had not been described. Through the courtesy of Mr. Allen McIntosh of the United States Bureau of Animal Industry, Beltsville, Maryland, I have been able to examine the two type specimens[2] upon which Manter's description was based and have found that a genital atrium does in fact exist (see Fig. 7). Consequently the species is truly a *Lecithophyllum*.

Lecithophyllum pyriforme should be excluded from this genus because of the difference in structure of the vitellaria.

Thus, four species, including the new one described herein, seem to constitute the genus *Lecithophyllum* at the present time.

Key to the Species of *Lecithopyllum*

1. Hermaphroditic pouch globular; prostatic duct four to five times its length..... ..... *L. intermedium* (Manter, 1934), n. comb.
2. Hermaphroditic pouch elongate; prostatic duct shorter than it.....3
3. Seminal vesicle entirely anterior to acetabulum; length of adults 2–3 mm..... ..... *L. botryophorum* (Olsson, 1868) Odhner, 1905
4. Seminal vesicle at least partially dorsal to acetabulum; length of adults less than 1.5 mm..5
5. Genital pore at level of mid-pharynx; group of three vitellaria anterior to and smaller than group of four..... *L. sphaerolecithum* (Manter, 1925) Manter, 1947
6. Genital pore at level of oral sucker; group of four vitellaria anterior to group of three and of equal sizes..... *L. anteroporum* n. sp.

In re-examining the type specimens of *L. sphaerolecithum* certain errors or omissions in Manter's account of this species, based on the 1.1 mm specimen, were noted. These are corrected in the following statements. Since Manter did not recognize a genital atrium, his description and measurements of the hermaphroditic pouch actually apply to this structure combined with the genital atrium. The hermaphroditic pouch measures 0.142 mm in length by 0.052 mm in maximum breadth and the genital atrium is 0.074 mm in length. Manter gave the length of the prostatic duct as about 0.19 mm but by measurement of the same structure is only 0.108 mm. The prostatic cells are not uniformly distributed about the prostatic duct but are arranged similarly to those in *L. anteroporum*. A short narrow duct joins the seminal vesicle and the prostatic duct. The posterior margin of the acetabulum is 0.53 (not 0.43) mm from the anterior end. The scale accompanying Manter's (15) Fig. 70 bears out this corrected measurement. The seminal vesicle is 0.158

[2]The 1.1 mm specimen is laterally mounted and the larger specimen is transversely sectioned.

mm in length by 0.098 mm in maximum breadth in lateral view. The post-vitelline body length is 0.209 mm. Measurements of 10 eggs selected at random ranged in size from 55.3 to 62.4 μ by 26.8 to 28.4 μ, with a mean of 58.9 μ by 27.5 μ. The oral sucker is 0.095 mm long and the ventral sucker 0.170 mm long, with a ratio of 1:1.78. The gonads and vitellaria are slightly smaller than indicated by Manter. These slightly smaller measurements of gonads, vitellaria, and suckers may be due to the fact that Manter's measurements were probably taken from a temporary ventral mount of the specimen and hence represent measurements along a different axis of these structures. The smaller egg dimensions are probably the result of shrinkage.

With the amended description of *L. sphaerolecithum* it is apparent that it is the closest relative of *L. anteroporum*, differing from this species as noted in the key. In addition, the seminal receptacle in fully mature individuals is about the same size as the ovary in *L. anteroporum* whereas it is only one-half the diameter of the ovary in *L. sphaerolecithum*. However, since the wall of the seminal receptacle is muscular its size may be largely dependent on the amount of sperm stored in it. The mean egg size of *L. anteroporum* appears to be smaller than that of *L. sphaerolecithum*, although there is considerable overlap in the ranges.

The lack of an adequate description of the terminal reproductive ducts in *L. botryophorum* precludes a detailed comparison of this species with *L. anteroporum*. Olsson's (24) illustration from the ventral aspect suggests that the ducts from the genital pore to the base of the seminal vesicle are linearly arranged, in contrast to the sigmoidal arrangement of these structures in *L. anteroporum* as seen in lateral view. However, a lateral view of *L. botryophorum* might be more revealing of the arrangement of the terminal genital ducts. Odhner (21) gave the length of the genital sinus (hermaphroditic duct) as 0.30 mm. Does this measurement actually refer to the hermaphroditic duct or is it the length of the sinus sac (hermaphroditic pouch), as interpreted by Dawes (2)? The hermaphroditic pouch is more readily measurable than the hermaphroditic duct, which lies coiled within the pouch, as in *L. anteroporum*. A true measurement of the length of the duct would be difficult to obtain unless it was completely extended, but its length must be at least equivalent to the combined lengths of the hermaphroditic pouch and the genital atrium. The length of this latter structure has not been cited. The need for clarification of the arrangement and lengths of the terminal genital ducts in *L. botryophorum* is thus apparent.

The Genera *Lecithophyllum*, *Aponurus*, and *Brachadena*

The genera *Aponurus* Looss, 1907 (11, 12) and *Brachadena* Linton, 1910 (9) are closely allied to *Lecithophyllum* Odhner, 1905 (21). Neither Looss nor Linton compared or attempted to differentiate their genera from *Lecithophyllum*. Subsequent attempts at differentiation and allocation of species within these genera have not been universally accepted. Odhner (23, footnote, page 6) noted that *Lecithophyllum* possesses a well-developed genital

atrium which is absent in *Aponurus*. Nevertheless he suggested that *Aponurus sphaerolecithus* Manter may actually belong to *Lecithophyllum* because of the large egg size, although it was not known at that time that a genital atrium is present in this species.

Manter (19) suggested that the two genera be separated on the basis of egg size and length of the hermaphroditic duct. He did not make use of the presence or absence of a genital atrium. *Lecithophyllum* was characterized as possessing a hermaphroditic duct longer than the pars prostatica and eggs 55 to 65 μ long. Species in which the egg size is less than 55 μ *or* in which the hermaphroditic duct is shorter than the pars prostatica were placed in *Aponurus*. Manter's concepts, which were accepted by Chauhan (3) and Skryabin and Gushanskaya (*in* Skryabin (26)), required the transfer of *A. sphaerolecithus* to *Lecithophyllum* and the transfer of *L. fuscum* to *Aponurus*. The discovery of *L. anteroporum* indicates that Manter's suggestions for distinguishing the two genera are impractical. In this species the hermaphroditic duct is longer than the pars prostatica but the egg length (38 to 61.6 μ) includes part of the range of *Lecithophyllum* as well as part of the range (up to 45 μ) of *Aponurus* species as cited by Manter.

Yamaguti (34), reverting in part to Odhner's (23) differentiation of the two genera, distinguished *Lecithophyllum* from *Aponurus* by the presence of a genital atrium and by the character of the hermaphroditic duct. For *Lecithophyllum* he described the duct as constricted into two portions, an enlarged basal portion and a dilated distal portion opening into the genital atrium, whereas in *Aponurus* it was described as tubular, without a constriction. A constriction of the hermaphroditic duct into two regions was not mentioned by Olsson (24) in his original description of *L. botryophorum*, the type species, nor in Odhner's (21) redescription of type material.[3] It therefore cannot be accepted yet as a generic character of *Lecithophyllum* in spite of the presence of this type of hermaphroditic duct in the other three species of the genus.

The essential character separating *Lecithophyllum* from *Aponurus* appears to be the presence of a well-developed genital atrium in the former genus and its absence in the latter one.

Yamaguti (34) relegated *Brachadena* to the synonymy of *Lecithophyllum*. However, the presence or absence of a genital atrium has not been established for *Brachadena*. Furthermore, the vitellaria in the type and only species, *B. pyriformis* Linton, 1910 (1, 9, 10, 13, 16, 19), have been described or illustrated by various authors (under this name or one of its synonyms as listed by Manter, 19) as consisting of seven elongate lobes united centrally. Olsson (24) originally described the vitellaria in *L. botryophorum* as composed of seven pear-shaped lobes united centrally, but, Odhner (22), in re-examining new material of this species, found that the vitellaria actually consist of two groups, one with three lobes and the other with four lobes, the lobes within

[3]Yamaguti (34) cited Linton's (7, 8) records of *L. botryophorum*. Linton (10) and Manter (16, 19) had already re-identified the specimens as belonging to no less than three species: *Lecithaster gibbosus*, *Leithaster confusus*, and *Brachadena pyriformis*.

each group being united. Looss (12) in his generic definition of *Aponurus* stated that the vitelline follicles are shaped like irregular spheres, frequently forming a group of three and a group of four. Later in the same paper he commented that in compressed fresh specimens sometimes the vitellaria are no longer recognizable as two distinct groups and the vitellaria then resemble those of *Lecithaster* since all lobes *seem* to originate at one point. He further wrote that (free translation) "I believe that this is indeed the case, even if the direct proof on mounted and cleared individuals cannot be produced because of the unfavourable position of the follicles." The use of the word 'seem' indicates uncertainty on Looss's part in interpreting the actual condition of the vitellaria in compressed specimens and he has presented no conclusive evidence that the vitelline lobes in *A. laguncula* are centrally united. Since the globular or subglobular vitellaria of *Aponurus* and *Lecithophyllum* are distributed in both depth and width of a cylindrical body, flattening of a specimen, particularly before fixation, considerably alters their shape and arrangement, the extent of this alteration depending on the amount of flattening. The vitelline lobes in a compressed specimen of *L. anteroporum* from *O. nerka*, although changed in shape and arrangement, do not give the impression of originating from a central point (Fig. 5). This was also borne out by study of temporary mounts of individual specimens, viewed ventrally, dorsally, laterally, or from various ventrolateral or dorsolateral aspects. Manter (15) also stated that he could find no evidence from serial transverse sections that the vitelline lobes of *L. sphaerolecithum* are centrally united. From the descriptions of the various species of *Aponurus* and *Lecithophyllum* it must be concluded that the vitellaria in these two genera consist of seven spherical to subspherical or short pyriform lobes which are not centrally united but which are frequently arranged in two groups, one of four lobes and the other of three lobes.[4] The members within each group, at least in *Lecithophyllum*, may be united.

On the basis of vitelline structure it is advisable, for the present, to retain *Brachadena* as an independent genus. Bravo-Hollis (1), in refuting Yamaguti's decision to unite *Brachadena* with *Lecithopyhllum*, tabulated several differences between them which seem to be only of a specific nature. She did not compare the structure of the vitellaria.

A provisional key to separate the three genera may be given as follows:

1. Vitellaria consisting of seven elongate lobes united centrally.....*Brachadena*
2. Vitellaria consisting of seven spherical, subspherical, or short pyriform lobes, not centrally united, but frequently arranged in two groups of three and four:
   a. Well developed genital atrium present.....*Lecithophyllum*
   b. Genital atrium lacking.....*Aponurus*

[4]*Aponurus vitellograndis* Layman has been described as having star-shaped vitellaria. The lobes, as illustrated by Layman (5), are pyriform, but much shorter than in *B. pyriformis*. This species may yet find its true position in *Brachadena* but the gross appearance of the vitellaria is considerably different from that in *B. pyriformis*. A reinvestigation of the vitellaria in *A. vitellograndis* should be undertaken before a decision is made. It may be discovered that the vitellaria are actually disposed in two groups. Skryabin and Gushanskaya (*in* Skryabin (26)) transferred Layman's species to *Hysterolecitha*, but one of the prominent characters of this genus is a tubular, winding, or coiled seminal vesicle which excludes *A. vitellograndis* from it.

As already noted, *Brachadena* is a monotypic genus and *Lecithophyllum* contains four species. The genus *Aponurus*, apparently the commonest of the three genera, has had 16 species assigned to it, of which one, *A. bowersi* Leiper and Atkinson, 1914 (6), does not belong to this genus because the vitellaria consist of two compact masses[5] and two others, *A. intermedius* and *A. sphaerolecithus*, have been transferred to *Lecithophyllum*. The remaining 13 species are *A. laguncula* Looss, 1907 (11, 12, 19, 33), *A. tschugunovi* Isaichikov, 1927 (4, 29), *A. vitellograndis* Layman, 1930 (5), *A. rhinoplagusiae* Yamaguti, 1934 (30), *A. brevicaudatus* Yamaguti, 1934 (30), *A. callionymi* Yamaguti, 1938 (31), *A. acropomatis* Yamaguti, 1938 (31), *A. breviformis* Srivastava, 1939 (28), *A. bengalensis* Srivastava, 1939 (28), *A. trachinoti* Manter, 1940 (18, 20), *A. argentini* Polyanskii, 1952 (25), *A. carangis* Yamaguti, 1952 (32), and *A. synagris* Yamaguti, 1953 (33).

The possibility of *A. vitellograndis* being a member of another genus has been noted earlier in this paper. The terminal reproductive ducts have been inadequately described and illustrated in *A. tschugunovi* so that its position within the genus cannot be verified. The description of the terminal genital ducts in *A. argentini* is not in accordance with the concept of *Aponurus*. Polyanskii (25) did not mention a hermaphroditic duct and pouch but rather described a cirrus and the uterus extending independently to the genital pore. Other characters are in agreement with the concept of *Aponurus* or *Lecithophyllum* and the description of the terminal genital ducts may be in error. In fact this species, which was taken from the same host (*Argentina silus*) and general locality as *L. botryophorum*, is identical with the latter in all respects except for the terminal genital ducts, and subsequent studies may prove the two species to be synonymous. All other species listed above have vitellaria consisting of seven spherical to short pyriform lobes, not centrally united, but commonly in groups of four and three, and a hermaphroditic duct enclosed in a pouch that starts immediately behind the genital pore.

In future morphological studies on members of the *Lecithophyllum–Aponurus–Brachadena* complex, particular attention should be focussed on the structure of the vitellaria and the terminal genital ducts in order to assist in clarification of the taxonomy of these genera.

References

1. Bravo-Hollis, M. Trematodos de peces marinos de aguas Mexicanas. XI. Estudio de 17 digeneos de las costas del Pacifico, incluyendo, seis especies nuevas y un genero nuevo. Anales inst. biol. México, **27**, 245–277 (1956).
2. Dawes, B. The Trematoda of British fishes. Ray Society, London. 1947.
3. Chauhan, B. S. Studies on the trematode fauna of India. Part IV. Subclass Digenea (Prosostomata). (A revision of Hemiuroidea from the Indian region.) Records Indian Museum, **51**, 289–393 (1954).

[5]Skryabin and Gushanskaya (*in* Skryabin (27)) transferred *Aponurus bowersi* to *Genolinea*. Since the seminal vesicle and details of the terminal genital ducts have not been described for this species, the transfer to *Genolinea* is unjustified. At the present time the correct generic allocation of *A. bowersi* must remain questionable.

4. ISAICHIKOV, I. M. On the parasitic worm fauna of fish of the family Mullidae. I. Parasitic worms from the Black Sea mullet *Mullus barbatus* L. (In Russian.) Trudy Sibir. Vet. Inst., Omsk, **9**, 117–132 (1927).
5. LAYMAN, E. M. Parasitic worms from the fishes of Peter the Great Bay. (In Russian with German summary.) Izvest. Tikhookeans. Nauch-Prom. Stantsii, **3**, 1–120 (1930).
6. LEIPER, R. T. and ATKINSON, E. L. Helminthes of the British Antarctic Expedition, 1910–1913. Proc. Zool. Soc. London, 222–226 (1914).
7. LINTON, E. Parasites of fishes of the Woods Hole region. Bull. U.S. Fish Commission for 1899, **19**, 405–492 (1901).
8. LINTON, E. Parasites of fishes of Beaufort, North Carolina. Bull. U.S. Bur. Fisheries for 1904, **24**, 321–428 (1905).
9. LINTON, E. Helminth fauna of the Dry Tortugas. II. Trematodes. Papers Tortugas Lab. Carnegie Inst. Wash. **4**, 11–98 (1910).
10. LINTON, E. Trematodes from fishes, mainly from the Woods Hole region, Mass. Proc. U.S. Natl. Museum, **88**, 1–172 (1940).
11. LOOSS, A. Zur Kenntnis der Distomen-familie Hemiuridae. Zool. Anz. **31**, 585–619 (1907).
12. LOOSS, A. Beiträge zur Systematik der Distomen. Zur Kenntnis der familie Hemiuridae. Zool. Jahrb. Abt. Syst. **26**, 63–180 (1907).
13. MACCALLUM, G. A. Studies in Helminthology. Zoopathology, **1**, 136–284 (1921).
14. MANTER, H. W. Some marine fish trematodes of Maine. J. Parasitol. **12**, 11–18 (1925).
15. MANTER, H. W. Some North American fish trematodes. Illinois Biol. Monographs, **10**, 1–138 (1926).
16. MANTER, H. W. Some digenetic trematodes of marine fishes of Beaufort, North Carolina. Parasitology, **23**, 396–411 (1931).
17. MANTER, H. W. Some digenetic trematodes from deep-water fish of Tortugas, Florida. Papers Tortugas Lab. Carnegie Inst. Wash. **28**, 257–345 (1934).
18. MANTER, H. W. Digenetic trematodes of fishes from the Galapagos Islands and the neighbouring Pacific. Repts. Allan Hancock Pacific Expedition, **2**, 329–497 (1940).
19. MANTER, H. W. The digenetic trematodes of marine fishes of Tortugas, Florida. Am. Midland Naturalist, **38**, 257–416 (1947).
20. MANTER, H. W. and VAN CLEAVE, H. J. Some digenetic trematodes, including eight new species from marine fishes of La Jolla, California. Proc. U. S. Natl. Museum, **101**, 315–340 (1951).
21. ODHNER, T. Die Trematoden des arktischen Gebietes. *In* Römer and Schaudinn, Fauna Arctica, **4**, 291–372 (1905).
22. ODHNER, T. *Stichocotyle nephropis* J. T. Cunningham ein aberranter Trematoden der Digenenfamilie Aspidogastridae. Kungl. Svenska Vetenskapsakad. Handl. **45**, 5–16 (1910).
23. ODHNER, T. Uber Trematoden aus der Schwimblase. Ark. Zool. Stockholm, 19A, **15**, 1–9 (1927).
24. OLSSON, P. J. Entozoa, Iakttagna hos skandinaviska hafsfisker. I. Platyhelminthes. Lunds Univ. Årsskr. **4**, 1–64 (1868).
25. POLYANSKII, I. I. Two new species of digenetic trematodes from fish of the North Atlantic. (In Russian.) Parazitolog. Sborn. Zool. Inst. Akad. Nauk. S.S.S.R. **14**, 266–280 (1952).
26. SKRYABIN, K. I. Trematodes of animals and man. Principles of trematodology. Vol. 9. (In Russian.) Akad. Nauk. S.S.S.R. Moscow. 1954.
27. SKRYABIN, K. I. Trematodes of animals and man. Principles of trematodology. Vol. 11. (In Russian.) Akad. Nauk. S.S.S.R. Moscow. 1955.
28. SRIVASTAVA, H. D. Two new parasites of the genus *Aponurus* Looss, 1907. Indian J. Vet. Sci. **9**, 31–35 (1939).
29. VLASENKO, P. V. Zur Helminthofauna de Schwarzmeerefische. (In Russian with German summary.) Trudy Karadag. Biol. Stantsii, **4**, 88–136 (1931).
30. YAMAGUTI, S. Studies on the helminth fauna of Japan. Pt. 2. Trematodes of fishes, I. Japan J. Zool. **5**, 249–541 (1934).
31. YAMAGUTI, S. Studies on the helminth fauna of Japan. Pt. 21. Trematodes of fishes, IV. Published by the author. Kyoto, Japan. 1938. 139 pp.
32. YAMAGUTI, S. Parasitic worms mainly from Celebes. Pt. 1. New digenetic trematodes of fishes. Acta Med. Okayama, **8**, 146–198 (1952).
33. YAMAGUTI, S. Parasitic worms mainly from Celebes. Pt. 3. Digenetic trematodes of fishes, II. Acta Med. Okayama, **8**, 257–295 (1953).
34. YAMAGUTI, S. Systema Helminthum. Pt. 1. Digenetic trematodes of fishes. Maruzen Co., Ltd., Tokyo, Japan. 1953.

ON THE LIFE HISTORY AND VALIDITY OF THE SPECIES IN PSOROPTES, A GENUS OF MANGE MITES[1]

GORDON K. SWEATMAN[2]

Abstract

The stages in the life cycle of *P. cuniculi* and other species or varieties in the genus are described. Host specificity currently attributed to the different types in *Psoroptes* is disproved, but differences exist in individual susceptibility. The six auricular types of psoroptic mites are reduced to two, and the five kinds of body mites to four. A dendrogram is included which shows the relations between the genera and species in Psoroptidae.

Introduction

Certain species of *Psoroptes*, like the sheep-scab mite, are pests of agricultural importance in various countries, and others, like those on wapiti, are a concern of wildlife biologists. It is well documented that psoroptic mites are more or less host specific, and the nine names in the genus are frequently descriptive of their hostal or geographical origin. Except for *P. natalensis*, all are so similar morphologically as to be currently considered indistinguishable. In the past, host specificity has been attributed not only to the types of *Psoroptes*, but also to other kinds of mange mites. Recently, however, Sweatman (37, 38, 40, 41) demonstrated that seven of the eight species or varieties in *Chorioptes*, and the three in *Otodectes* are biologically and morphologically identical. All three genera are close relatives in the same family. A review of the literature suggested that the validity of the host specificity and hence the names of psoroptic mites were based on limited experimental observations and needed verification. Before this was possible, however, it was imperative to obtain psoroptic mites from as many hosts and geographical centers as possible and compare them morphologically. Although the diagnostic features necessary to separate *Psoroptes* from related genera can be found in a variety of publications, there is, strange as it may seem, no adequate description of a single species in the genus. In the current study, therefore, there is included a morphological description of the various stages in the life cycle of *P. cuniculi* and *P. equi* var. *caprae* followed by a comparison of other types in the genus with these as a standard. Following the presentation of new data and a summary of the old, this paper shows that the genus is oversplit.

I. Morphology and Life Cycle

A. DESCRIPTION OF STAGES

Psoroptic mange mites are obligatory, non-burrowing parasites with chelicerae that pierce and chew the superficial skin of the host, causing inflammation

[1]Manuscript received April 1, 1958.
Contribution from the Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, in co-operation with the Institute of Parasitology, Macdonald College P.O., Que., Canada.

[2]Former address: Animal Pathology Laboratories, Health of Animals Division, Canada Department of Agriculture, Montreal Area Branch Laboratory, Macdonald College. Present address: Hydatid Research Unit, Medical School, University of Otago, Dunedin, New Zealand.

and edema at the site of infestation. According to the early writers Delafond and Bourguignon (in ref. 1), the life cycle of *P. ovis* contained immature mites that molted several times. Gerlach (8) disputed this, believing that the egg and larva were followed by an eight-legged mite that matured without molting. Mégnin (22), Henry (9), and others included in the life cycle the egg, larva, nymph, pubescent female or "first female adult stage", ovigerous female, and adult male. If the data are presented in this fashion, it can be interpreted that there is but one nymph which does not show sexual dimorphism and precedes either the pubescent female or adult male. This scheme gives the female two adult stages, and the male cycle one stage less than the female cycle. Shilston (35), Downing (6), and Lucas (18), each of whom used an isolation unit on the sheep to confine one or a few mites at a time, conformed to this life cycle, and, additionally, pointed out that a quiescent period preceded each motile stage. More recently, however, Priselkova (29) recognized two morphologically distinct nymphal stages not only in the female but also in the male cycle of *P. ovis*.

The present writer determined the life cycle of *P. cuniculi* and *P. equi* var. *caprae*, the ear mites from the rabbit and goat. These mites could not be reared in vitro using the technique described (38) for *Chorioptes bovis* and *Otodectes cynotis* (perhaps because of differences in feeding), but it was possible to incubate the eggs and quiescent stages at 35° C and 80% R.H. Larvae emerged from the eggs, and from vials containing individual quiescent mites, it was possible to mount on a microscopic slide the exuvium and emergent mite. From an examination of these, the developmental sequence and morphological changes from one stage to the next were ascertained. *P. cuniculi* and *P. equi* var. *caprae*, like *Chorioptes bovis* and Priselkova's report on *P. ovis*, contain the same number of stages in both the male and female cycles. They are the egg, larva, protonymph, deutonymph, and adult. These terms will be used in this paper for the male cycle, but in the female cycle the designate for the deutonymph and adult will be pubescent female and ovigerous female. Writers on *P. ovis* have stated that the pubescent female stage occurs in attachment for two or more days with the adult male forming the copulation pair. The current writer prefers the term attachment pair, since in *Chorioptes bovis* and *Otodectes cynotis*, it was demonstrated that the adult male copulates with the ovigerous female at a precise moment during her final molt, and not during the preceding long period of attachment.

All stages of *P. cuniculi* and *P. equi* var. *caprae* are indistinguishable. The structural nomenclature used in the description below is identical with that used previously for *C. bovis* (38), and measurements are given in microns with the mean and standard deviation of individuals (measured 15) in the first and second parentheses respectively.

Larva (Fig. 1)

An elongate oval mite with moderately short legs. It is soft-bodied and grayish brown in color. No sexual dimorphism was noted.

Dorsum

Length 287–399 (322) (31) and width 177–254 (220) (21). Sclerotized rectangular propodosomal plate with a pair of short propodosomal plate setae near the posterior corners. Remainder of integument finely striated. One pair of quite long dorsal setae arise posterolaterally to the propodosomal plate setae. Two pairs of dorsolateral setae occur, the lateral propodosomal being shorter than the lateral metapodosomal. Five pairs of short idiosomal setae occur.

Venter

Integument finely striated. Only four pairs of setae present: one propodosomal, two metapodosomal, and one long pair of terminal opisthosomal.

Legs

Legs I and II with five articles each, ambulatory, and approximately the same length, being 126–147 (133) (6) and 126–145 (133) (6) respectively.* Leg III with six small, weak articles, mainly tactile, and measuring 76–85 (82) (3).† Coxae of all legs specialized as apodemata. Setae on legs I and II both long and moderately short, and increase numerically on distal articles—absent on trochanters; femurs I and II each with a single seta; genua with two setae each; tibiae I and II with one seta and one fairly stiff rod each, the latter situated anterodorsally; tarsus I with seven setae, one moderately long rod in the distodorsal position, and a special pit, possibly a sensory structure, in the proximodorsal position; and tarsus II with five long setae and a moderately short rod in the proximodorsal position. Tarsi I and II each with a single claw and a long jointed pedunculated caruncle measuring 39–52 (43) (4). Pretarsi absent. Yunker (45) believed the first joint of the peduncle supporting the caruncle to be the pretarsus. The present writer does not conform to this, but believes the pretarsus to be absent, since, in the close relative *Chorioptes bovis*, the pretarsus occurs as a "thumblike" protuberance from the tarsus and is a distinct entity from the peduncle supporting the caruncle.

Leg III, the subdistal article of which has a moderately long seta and a short rod. The distal article has two short setae and terminally two extremely long whiplike setae of which the posterior is longer than the anterior.

Gnathosoma

Chelicerae dorsal measuring 56–74 (66) (5), taper apically, moderately chelate with the movable digit articulating dorsoventrally by its posterior extremity in a cleft of the fixed digit; each with three pointed teeth. Figured for *P. equi* by Buxton (2).

Pedipalpi located at the lateroventral surface of the gnathosoma. Possess three pairs of articles which gradually shorten and taper distally, the terminal article ending in a number of lappets. Each article has a single seta. The hypostome has one pair of moderately long, centrally located setae. The gnathosoma is essentially the same in all stages, except for an increase in relative size.

*Measured from coxa along the anterodorsal border. Does not include caruncle.
†Measurement of only the articulating part of the leg along the outer surface.

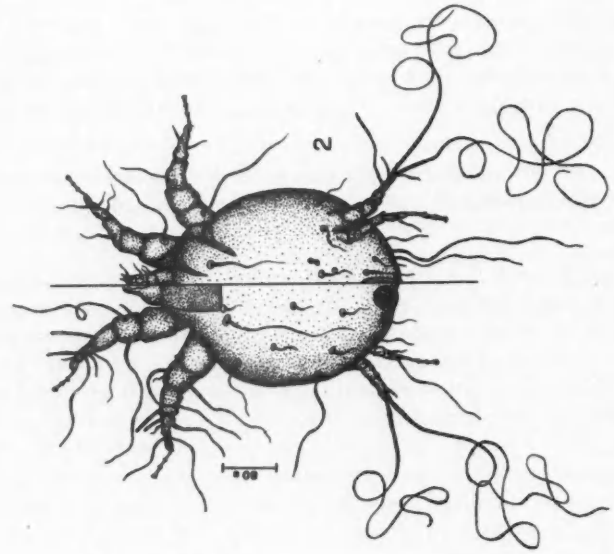


FIG. 2. Dorsoventral view of female protonymph.

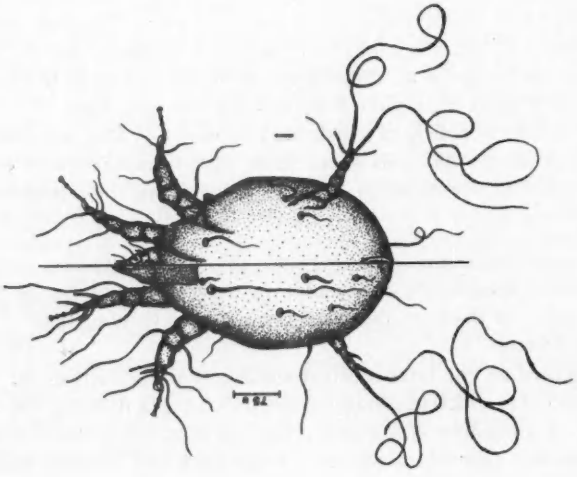


FIG. 1. Dorsoventral view of larva.

Male Protonymph

Idiosoma and gnathosoma much the same as in larva, except that the protonymph is larger, possesses additional setae, and adds the fourth pair of legs. Essentially the same as female protonymph (Fig. 2) except for the absence of the posterior suckers.

Dorsum

Length 303–419 (353) (34) and width 242–319 (266) (22). Possesses propodosomal plate and same setae as larva with one additional pair of short idiosomal setae in the medioterminal position.

Venter

The four pairs of setae in the larva occur in this stage; together with an additional pair of metapodosomal setae and four pairs of opisthosomal setae.

Legs

Leg I 154–182 (167) (9); leg II 156–174 (165) (6); leg III 101–121 (109) (5); and leg IV* 84–109 (93) (7) long. Caruncles I and II 45–61 (53) (4). In addition to the features described already for the larva, tarsus II has a short seta immediately anterior to the posterodorsal rod; and genua I and II, each of which has a short, inconspicuous seta located immediately in front of the long dorsal seta. Sometimes, however, this short seta is displaced laterally and more rarely posteriorly to the dorsal seta. Leg IV is composed of six articles that taper apically. The terminal article possesses one long and two short setae and a long jointed pedunculated caruncle.

Gnathosoma

Essentially as in larva, except for increase in size. Chelicerae 76–85 (80) (3).

Female Protonymph (Fig. 2)

This stage is essentially the same as the male protonymph, with the additional feature of a pair of posterodorsal suckers.

Dorsum

Length 293–420 (346) (41) and width 242–370 (289) (45). Setae identical with male protonymph. A pair of suckers occurs at the tip of the opisthosoma.

Venter

Identical with that of the male protonymph.

Legs

Leg I 155–180 (165) (8); leg II 158–185 (166) (8); leg III 97–113 (105) (4); and leg IV 81–93 (87) (4) long. Caruncles I and II 48–61 (52) (5). Setae identical with those on male protonymph. In one instance, an anomaly was observed where the distal article on leg IV possesses an additional short seta which is a regular feature on the pubescent female and male deutonymph.

Gnathosoma

As for preceding stages. Chelicerae 72–85 (78) (4).

*Measurement of the articulating leg along the inner surface.

Male Deutonymph

Immature characters persist in this stage, with the addition of two pairs of ventral metapodosomal setae, an extra pair of setae on leg III and two on leg IV, one pair of long setae on trochanters I and II, and a pair of rods on tarsus I. Essentially the same as the pubescent female (Fig. 3) except for the absence of posterior suckers.

Dorsum

Length 370–547 (455) (62) and width 274–380 (331) (34). Morphological features identical with those of male and female protonymph.

Venter

Five metapodosomal setae on this mite compared with three on the protonymph distinguish the two stages.

Legs

Leg I 163–204 (185) (15); leg II 163–209 (185) (15); leg III 129–145 (136) (7); and leg IV 118–147 (109) (10) long. Caruncles I and II 52–68 (59) (6). Trochanters I and II have developed one long seta each, while the femur, genu, and tibia remain identical with the preceding stages. Tarsus I, but not tarsus II, however, has an anterior rod in addition to the structure on the protonymph. The proximal articulating article on leg III has acquired a moderately long seta, but the rest of the leg remains unchanged. Leg IV has two additional setae, one on the apical article and the other on the subterminal article.

Anomalies are not uncommon among invertebrates. On one male deutonymph from the rabbit, the distal part of tarsus II possesses a stunted peduncle supporting an equally stunted caruncle as well as a double rather than single-barbed hook (Fig. 7). Tarsus II on the opposite leg is normal.

Gnathosoma

As for preceding stages. Chelicerae 79–90 (82) (3).

Pubescent Female (Fig. 3)

Dorsum, venter, gnathosoma, and legs identical with the male deutonymph with the addition of a pair of dorsoposterior suckers; or dorsum same as on female protonymph except for difference in size.

Dorsum

Length 346–670 (436) (84) and width 316–435 (353) (36). Morphological features identical with female protonymph.

Venter

Identical with that of the male deutonymph.

Legs

Leg I 145–184 (163) (13); leg II 137–182 (168) (13); leg III 77–109 (94) (11); and leg IV 77–111 (89) (11) long. Caruncles I and II 35–56 (45) (7) long. Morphological features identical with male deutonymph.

Gnathosoma

As for other stages. Chelicerae 77–97 (83) (5).

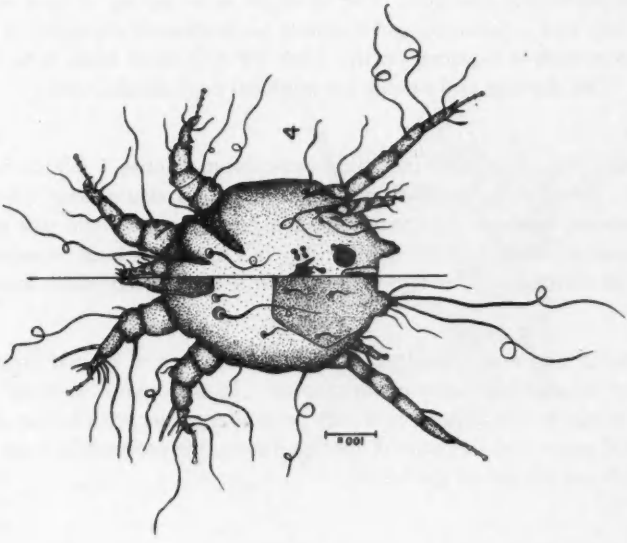


FIG. 4. Dorsoventral view of adult male.

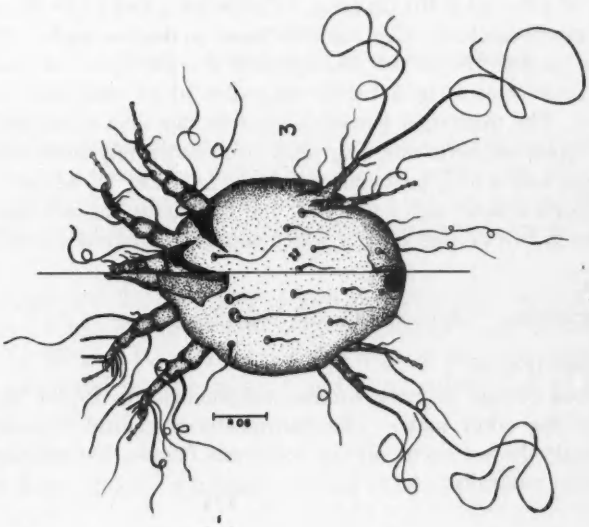


FIG. 3. Dorsoventral view of pubescent female.

Adult Male (Fig. 4)

Transformation from the male deutonymph to the adult is marked by distinct morphological changes. The whiplike setae on leg III are replaced by two prongs and a caruncle, and the long pedunculated caruncle on leg IV of the deutonymph is replaced on the adult by two small lobes with minute caruncles. The dorsum and venter are modified considerably also.

Dorsum

Length 431–547 (472) (29) including opisthosomal lobes. Width 322–462 (406) (36). Besides the propodosomal plate there is also a large sclerotized area that covers much of the hysterosoma. Idiosoma with one pair of setae less than deutonymph, but the adult male has a pair of large opisthosomal lobes each of which supports two long and three moderately short setae.

Venter

Just anterior and lateral to the anus, there is a pair of adanal copulatory suckers with an associated pair of short setae. In the central metapodosomal region the reproductive apparatus is now present together with a pair of setae. The perianal setae and one pair of metapodosomal setae which occur on the deutonymph are absent on the adult.

Legs

Leg I 213–269 (243) (15); leg II 214–274 (251) (16); leg III 293–412 (351) (26); and leg IV 105–132 (116) (8) long. Caruncles I and II 69–85 (81) (5). Legs I and II morphologically identical with those on deutonymph. The long whiplike setae on leg III of the deutonymph do not occur on the adult, and this leg transforms from a tactile structure to an essentially ambulatory structure. The trochanter possesses one seta, the tibia a long seta and a rod, and the tarsus supports one long seta, two moderately short setae, two terminal prongs, and a long pedunculated caruncle. Leg IV has six articles. The tibia supports a short seta and a rod, and the tarsus has two short setae and terminates in two lobules each of which supports a minute caruncle.

Gnathosoma

As for other stages. Chelicerae 81–108 (96) (9).

Ovigerous Female (Fig. 5)

The ovigerous female is more similar morphologically to the immature stages than is the adult male. The dorsoposterior genital suckers of the pubescent female do not occur on the ovigerous female, but the latter has acquired a vulva ventrally.

Dorsum

Length 403–749 (568) (98) and width 351–499 (411) (54). Setal pattern identical with pubescent female, but the posterior genital suckers of the latter have been lost.

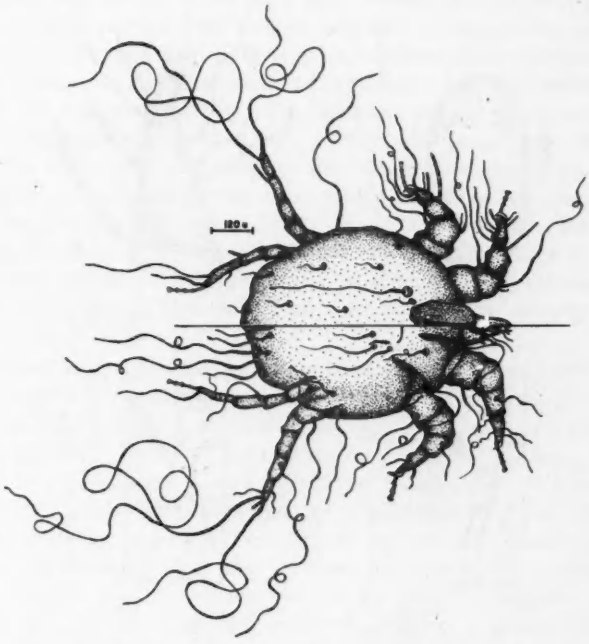


FIG. 5. Dorsoventral view of ovigerous female.

Venter

The vulva consists of a transverse slit and is located in the posterior propodosomal region. Generally three (Fig. 8), but sometimes only two (Fig. 9) pairs of setae are located behind the vulva. Only two pairs of metapodosomal setae occur compared with five pairs on the pubescent female.

Legs

Leg I 200–311 (241) (40); leg II 209–322 (248) (42); leg III 158–277 (212) (52); leg IV 180–270 (205) (36) long. Caruncles I and II 74–100 (84) (7). Legs I, II, III, and IV are identical with those of the pubescent female except for an additional seta on tarsus I and short rod on each of tarsus III and tarsus IV.

Gnathosoma

As for other stages. Chelicerae 97–158 (124) (23).

Egg

The egg is elliptical and has a white, shiny surface. The shell has two bosses on the same side and towards one end of the egg (Fig. 6). Egg cleavage is along the longitudinal axis. Length 190–274 (228) (29).

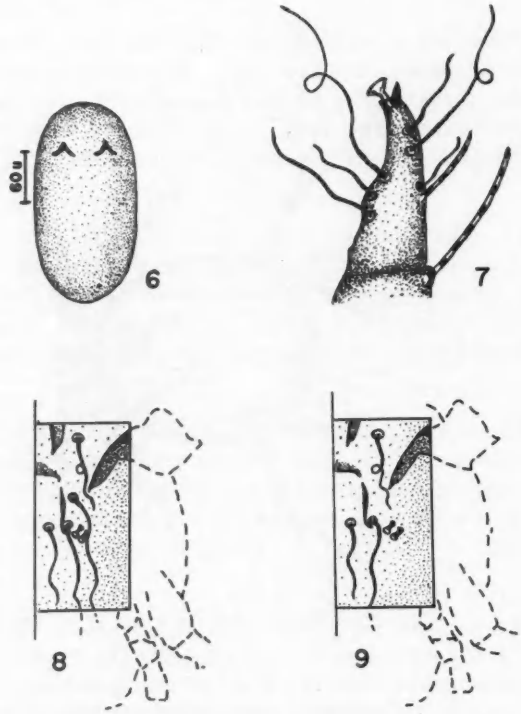


FIG. 6. Egg shell with two bosses. FIG. 7. Malformation of tarsus II of a male deutonymph. FIGS. 8 and 9. Three (Fig. 8) and two (Fig. 9) setae in the region of the vulva on the ovigerous female.

B. LIFE CYCLE

The time required for a stage in the life cycle of *P. ovis* has been reported by a number of authors. Eggs on the skin of the sheep are reported as hatching after a period of 1 to 3 days (6), 2 to 3 days (20, 35), and 3 to 4 days (8, 36). If separated from the skin by scab or debris, Downing (6) observed 4 to 5 days were required, and in tags of wool a few inches from the hide, egg incubation took up to 10 days. Shilston (35) observed that 6 to 8 days were needed to incubate eggs 2 inches from the skin, and Stockman and Berry (36) stated that the usual egg incubation period was 4 to 6 or 7 days. Gillette (in ref. 1) carried eggs in a vial in his pocket that required 5 to 9 days to hatch, and Priselkova (29) observed that eggs at 36–37° C and a relative humidity above 85% hatched from 78 to 88 hours. These observations suggest that the period of incubation of the eggs is affected directly by extrinsic factors. The validity of some of the minimum periods recorded is questionable since the eggs were obviously collected at random and no doubt had already undergone some incubation before being placed on experiment.

Writers on *P. ovis* gave the larval period as 2 to 4 days, and this was said to be followed by a 3- to 4-day nymphal stage. Downing (6), however, observed that some nymphs persisted for 6 days, and these emerged into adult males. The adult male was then said to become attached with the pubescent female which molted into an ovigerous female 2 days later. Another day was required before the first egg was laid (6). The complete egg-to-egg cycle (minimum period?) has been given as 9 days (35), 10 days (18), or 10 to 12 days (6, 13, 36). Kemper and Roberts (16) stated that the females deposited eggs when approximately 10 to 12 days old, presumably not including the incubation time of the eggs which they gave as 4 to 7 days. On this basis, the period for the life cycle would be 14 to 19 days. Priselkova (29) noted that the complete cycle of *P. ovis* took 17 to 18 days under optimal conditions (probably 36–37° C and at least 85% R.H.), but from 1 to 3 months at low temperatures and relative humidities.

In the current study, eggs and quiescent mites were collected at random from the ears of rabbits (*P. cuniculi*) and goats (*P. equi* var. *caprae*) and placed in a series of vials which were then held under suitable conditions (35° C, 80% R.H., and complete darkness) for incubation or transformation. Replicate trials showed that eggs took up to 4 days to hatch and quiescent mites up to 30 hours to molt. These are considered the approximate periods required for these processes under the physical conditions outlined, and the shorter periods observed are invalidated since the parasites had no doubt undergone some development while still on the host before they were collected. The total time for the egg incubation and three quiescent stages in a single cycle would be 7 days, the same as for *Chorioptes bovis* (38). For a complete cycle, additional days would be required for the three immature motile stages and preoviposition period of the ovigerous female. Undoubtedly, the motile periods would be longer than the quiescent periods, and it is possible that *P. cuniculi* and *P. equi* var. *caprae*, like *C. bovis*, would require about 3 weeks for a complete cycle under the above conditions.

II. Non-Specificity and Revision of the Genus *Psoroptes*

Nine species or varieties of *Psoroptes* have been described. Most have been found on farm stock, but other hosts include the gazelle, Indian water buffalo, bighorn, and wapiti. Before the experimental results are described, it is imperative to review the taxonomic status and morphological criteria used in the past to distinguish the types of *Psoroptes*. Authority has dictated that psoroptic mites are more or less host specific and therefore distinct physiologically even though most are indistinguishable morphologically. In 1922, Hirst (11) attempted to locate differentiating morphological characters on mites from different hosts, and pointed out that the setae on the opisthosomal lobes of the adult male were of some diagnostic value. The current writer confirms that this is the only body area that has reasonably reliable characters for distinguishing some types, and reports additional features beyond those noted by Hirst.

Because there has been so much discrepancy in the names used for these parasites, it is necessary to preface this section with a list of the names and when first adopted by the various authors. These are given below according to host and whether they are body mites or auricular mites.

GENUS: *Psoroptes* Gervais, 1841

TYPE SPECIES: *Sarcoptes equi* Hering, 1838

1. Body Mites

Horse, donkey, mule.—*Sarcoptes equi* Hering, 1838; *Psoroptes equi* Gervais, 1841; *Dermatodectes equi* Gerlach, 1857; *Dermatodectes communis* Delafond, 1859; *Dermatokoptes communis* Fürstenberg, 1861; *Psoroptes longirostris* var. *equi* Mégnin, 1877; *Psoroptes communis* var. *equi* Railliet, 1885; *Psoroptes equi* var. *equi* Neveu-Lemaire, 1938.

Domestic sheep.—*Sarcoptes ovis* Hering, 1838; *Psoroptes ovis* Gervais, 1841; *Psoroptes longirostris* var. *ovis* Mégnin, 1877; *Psoroptes communis* var. *ovis* Railliet, 1893; *Psoroptes equi* var. *ovis* Neveu-Lemaire, 1938.

Domestic cow.—*Dermatodectes bovis* Gerlach, 1857; *Psoroptes longirostris* var. *bovis* Mégnin, 1877; *Psoroptes communis* var. *bovis* Railliet, 1893; *Psoroptes bovis* Canestrini and Kramer, 1899; *Psoroptes equi* var. *bovis* Neveu-Lemaire, 1938.

Domestic cow and Indian water buffalo (*Bubalus bubalis*).—*Psoroptes natalensis* Hirst, 1919.

Bighorn (*Ovis canadensis*).—*Psoroptes ovis* (see Hall in Ward, 1915 (44); Seton, 1929 (34); and Honess and Winter, 1956 (12)).

Wapiti (*Cervus canadensis*).—*Psoroptes communis* var. *cervinae* (identified by Dr. E. W. Price and reported in Murie, 1951 (25)); *Psoroptes equi* var. *cervinae* (identified by Dr. G. W. Wharton and reported in Honess and Winter, 1956 (12)).

2. Ear Mites

Domestic rabbit.—*Dermatodectes cuniculi* Delafond, 1859; *Psoroptes longirostris* var. *cuniculi* Mégnin, 1877; *Psoroptes communis* var. *cuniculi* Railliet, 1893; *Psoroptes equi* var. *cuniculi* Neveu-Lemaire, 1938.

Domestic and wild goat.—*Psoroptes communis* var. *caprae* Railliet, 1893; *Psoroptes congolensis* Mense (?) (synonymized by Gedoelst, 1909 (7)); *Psoroptes equi* var. *caprae* Neveu-Lemaire, 1938.

Gazella sp.—*Psoroptes gazellae* Canestrini, 1894.

Bighorn.—*Psoroptes cervinae** Ward, 1915; *Psoroptes communis* var. *cervinae* Hirst, 1922; *Psoroptes equi* var. *ovis* (identified by Dr. G. W. Wharton and reported in Honess and Winter, 1956 (12); see also Mills, 1937 (23)).

Horse, donkey, mule.—*Psoroptes hippotis* Henry and Railliet, 1920 *in* Henry, 1920 (9).

Domestic sheep.—*Psoroptes ovis* (see Railliet, 1893 (30); and Hirst, 1922 (11)).

*Ward (44) possibly chose *cervinae* to be euphonious with *caprae* and *gazellae*, but the older names can be used as Latin substantives in the genitive, whereas *cervinus*, being only an adjectival form, cannot be used in the genitive since the nomenclatural rules state that an adjectival specific name is to be used in the nominative singular. Since *Psoroptes* is masculine (of Greek derivation), *cervinae* is hereinafter emended to *cervinus* to conform to this rule.

Individual versus Species Susceptibility

Just because a parasite is transferred more readily between different individuals in the same host species than between different species is not adequate basis for giving the parasite on the new host a distinct specific, or even varietal, name. When Sweatman (38) synonymized seven of the eight species or varieties of *Chorioptes*, considerable data were presented which demonstrated that some individuals among many species of ungulates were apparently susceptible to *C. bovis* while other individuals in the same species were partially or totally refractive. This well-recognized principle has also been shown to apply to *Otodectes* (41), another member of Psoroptidae. There is already some published evidence for this with regards to *Psoroptes*. Transfer of *P. cuniculi* from rabbit to rabbit was readily accomplished by early workers (see ref. 30), but Lucet (20) observed certain individuals refractive to infestation. Babcock and Black (1) stated that sheep infested with *P. ovis* and maintained under identical conditions did not show the same degree of scabbiness or irritation, and some sheep exposed continually to infestation never shed wool. In the current study, results at autopsy showed that one of four goats could not be infested with psoroptic ear mites. Another goat and a sheep had heavy infestations in one ear while the other ear remained uninfested. There appear therefore to be distinct differences in individual susceptibility among the host species.

Morphological Comparisons of the Types of Psoroptes

Hirst (11) did not conform to Canestrini and Kramer's (4) designation of specific rank for the types of *Psoroptes*, but proposed that in this genus there was *P. natalensis* with its narrow, spatulate setae on the ends of opisthosomal lobes of the adult male (Fig. 15), and since all other forms were morphologically indistinguishable that they be considered races of *Psoroptes communis* (a name proposed by Railliet (30) and later corrected to *Psoroptes equi* by Neveu-Lemaire (27)), with each virtually unique to its own host and given a distinct varietal name. In order to evaluate the concept of the absence of distinguishing morphological features between most types, the present writer obtained psoroptic mites from a variety of hosts and geographical centers for comparison. The geographical locations and hosts were: domestic rabbit (England, France, Cyprus, South Africa, United States, Ontario, Quebec); domestic goat (South Africa, New England states, Quebec); bighorn (Wyoming, Idaho, Colorado); wapiti (Wyoming); horse (England, France, Egypt, South Africa); domestic cattle (New Zealand, Australia, South Africa, New Mexico, Texas); and domestic sheep (England, Scotland, South Africa).

It was noted previously that all stages of the ear mites of the rabbit (*P. cuniculi*) and goat (*P. equi* var. *caprae*) are indistinguishable. The outer of the three setae at the end of the opisthosomal lobe of the adult male (hereinafter referred to as the "outer opisthosomal seta") is short (Fig. 10), and, on a few individuals from both hosts, there is sometimes a thumblike process situated laterally to the outer opisthosomal seta (Fig. 11). This seta on *P. cuniculi*

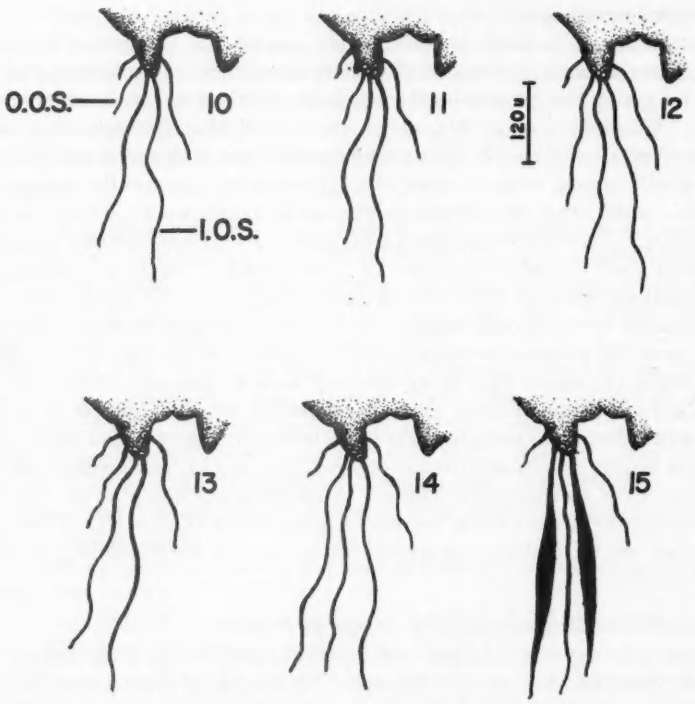


FIG. 10. Setae on opisthosomal lobe of adult male of the ear mite from rabbits and goats: O.O.S. = outer opisthosomal seta; I.O.S. = inner opisthosomal seta. FIG. 11. Setae and lappet on opisthosomal lobe of adult male of the ear mite from rabbits and goats. FIGS. 12–15. Setae on opisthosomal lobe of adult males of *P. cervinus* (Fig. 12); of body mite of sheep, cattle, and horses (Fig. 13); of body mite of horses (Fig. 14); of *P. natalensis* (Fig. 15).

and *P. equi* var. *caprae* is, however, quite distinct from the same seta on the ear mite (*P. cervinus*) of bighorn (Fig. 12). The former has an average length of 89 μ, while that of *P. cervinus* is 204 μ (Fig. 16). They are statistically distinct, although a few (2 of 97) in the upper range of *P. cuniculi* and *P. equi* var. *caprae* overlap the lower extreme of *P. cervinus*. Figure 16 shows that this measurement on mites from the different hosts results in a skew, rather than a normal distribution demonstrating that an occasional seta is "extra long". Clearly, in almost all cases, the adult males of *P. cuniculi* and *P. equi* var. *caprae*, although indistinguishable from each other, are distinguishable morphologically from *P. cervinus*. All other stages in *P. cervinus* are identical with those of *P. cuniculi*.

The body mites *P. ovis*, *P. bovis*, and *P. equi* generally have been considered indistinguishable, although Palimpsestov (28) published a complicated key to separate the larvae of these three species. The validity of the criteria used in the key was questioned by Priselkova (29), and the current writer could find

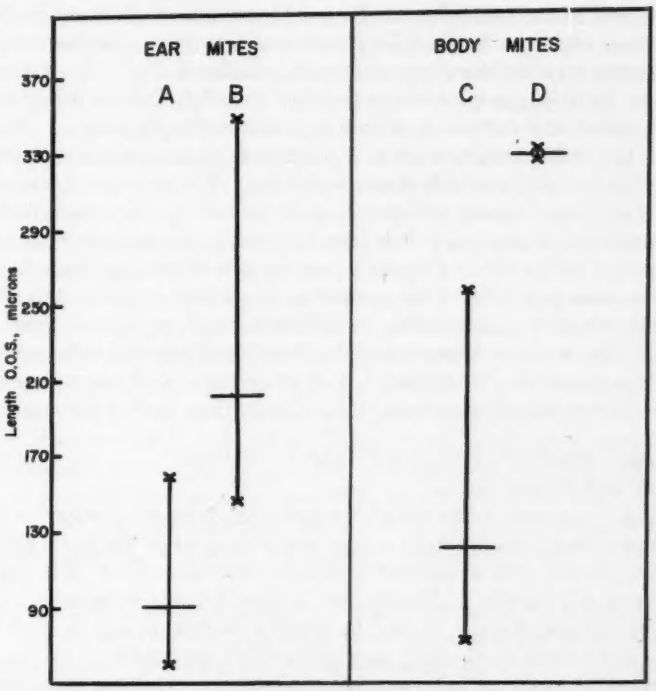


FIG. 16. Comparative length of the outer opisthosomal seta on adult males from different hosts. The vertical line marks the range, and the horizontal bar the mean.
A = ear mite of the rabbit and goat (n = 97).
B = ear mite of the bighorn (n = 45).
C = body mite of the sheep, cow, and horse (n = 112).
D = body mite of the horse (n = 3).

no consistently reliable character among those raised for larvae by Palimpsestov or on any other stages of *P. ovis*, *P. bovis*, and some of the mites from horses. On these mites the measurements of the length of the outer opisthosomal seta fell within the same range and are combined in Fig. 16. Another sample of horse mites has outer opisthosomal setae that are distinctly longer, but only three were available* for study. The average length of the seta is 303 μ, while on the former group it is 122 μ (Fig. 16). Both kinds of horse mites originated in England, but were from different farms. Perhaps only one of these is *P. equi*, while the other is a different type. For the purposes of this paper, the mites with the long outer opisthosomal setae (Fig. 14) are arbitrarily considered as the true *P. equi*.

On the basis of South American material, Rocha *et al.* (31) suggested that *P. natalensis* be accepted as a synonym of *P. bovis* since both produce mange on

*These specimens were obtained from the collection in the British Museum of Natural History.

the same host at the same sites (tailbase and withers); both occur in the same geographical region of South Africa; and since the outer and inner spatulate opisthosomal setae on the adult male of *P. natalensis* (Fig. 15) are sometimes so narrow as to suggest a broad variation in width from a threadlike type (as in *P. bovis*) to a definite spatulate type (as in *P. natalensis*). This writer confirms that the spatulate setae of *P. natalensis* are sometimes so narrow that it would be justifiable to call them threadlike. The synonymy suggested by Rocha *et al.* (31) is considered unacceptable, however, since the length of the outer opisthosomal seta of *P. natalensis* is always about as long as the inner opisthosomal seta, while in *P. bovis* it is about a third as long (Figs. 15 and 13). When the outer and inner opisthosomal setae are both about equally long and threadlike, then it is impossible to differentiate *P. natalensis* from *P. equi* (as designated herein). Since a mixed infestation is possible, only experimental research will clarify the validity of *P. natalensis*, and for the present *P. natalensis* is considered morphologically distinct from both *P. bovis* and *P. equi*.

*Biological Comparisons between the Types of Psoroptes**

A. Auricular Mites

Auricular psoroptic mites have been separated from one another on the basis of the hosts they infest rather than on morphological or biological differences. There are currently six recognized species or varieties. They, with their hosts, are *P. cuniculi* (rabbit); *P. equi* var. *caprae* (goat); *P. gazellae* (*Gazella*); *P. hippotis* (horse, donkey, mule); *P. cervinus* (bighorn); and *P. ovis* (domestic sheep). The validity of these species required verification. In the current study, direct transfer of mites was made from rabbits to four of six goats that originated from the same herd. At autopsy 3 to 4 months later, three of the four experimental goats contained reproducing mite populations in their ears, while the two control goats remained negative. Mites from these two hosts are therefore biologically indistinguishable, and since it was pointed out previously that their life histories are identical and that they are morphologically indistinguishable, it is suggested that *P. equi* var. *caprae* and *P. cuniculi* are conspecific. The name with priority is *P. cuniculi*.

P. ovis has been reported from the ears of domestic sheep without occurring on the body (11, 42), and it has been suggested that the ears serve as a source of infestation for the body and should receive particular attention during treatment. It seemed possible to the present writer that the sheep ear mite may be *P. cuniculi* rather than *P. ovis*. With this in mind, 12 Suffolk lambs from the same flock were divided into three equal groups. On two or three

*In addition to the hosts discussed, Seton (34) refers to body scab on American bison (*Bison bison typicus*), and Garretson writes (in ref. 32): "Mange was the most common disease of the buffalo, and when infested animals were found by the Indians, (they) were killed to the last individual . . ." If this scab were caused by psoroptic mites (sarcoptic mites would be the other likely possibility), then the geographical location suggests that transfer may have been possible from wapiti, bighorn, cattle, sheep, or horses.

Vitzthum (43) stated that he collected *Psoroptes*-like mites from the ears of a cheetah (*Acinonyx guttatus*) of unknown origin. As Vitzthum pointed out, the parasite occurred in a felid where one would have expected to find *Otodectes*. The chelicerae were described as being long and pointed like *Psoroptes* in contrast to the short chewing chelicerae of *Otodectes*.

occasions, auricular mites were transferred* from rabbits to one group; from goats to the second group; and the third group served as a control. When the animals were autopsied during the following 5 months, all eight experimental lambs were infested, while the control animals remained negative. Additionally, another lamb from the original flock was corralled with positive goats. The ears of this lamb were positive at autopsy, demonstrating transfer by direct bodily contact. These results extend the host range of *P. cuniculi* to include domestic sheep; further the evidence for *P. equi* var. *caprae* being the same as *P. cuniculi*; and indicate that sheep may be infested with two psoroptic species, namely *P. cuniculi* and *P. ovis*.

The above does not preclude the possibility of *P. ovis* being an ear mite as well as a body mite. There is evidence to clarify this. Shilston (35) was unable to transfer body mites from sheep to the ears of goats or rabbits either manually or from bodily association, and Delafond and Bourguignon (in ref. 11) before him could not transfer sheep mites to goats. There is little doubt that the ear mite *P. cuniculi* is distinct from *P. ovis* since, in countries like New Zealand, Australia, and Canada, sheep scab is virtually eradicated, while mites are a common occurrence in the ears of rabbits and goats. When combined, these data indicate that *P. ovis* is a body rather than an ear mite, and reports of this species in the ears of domestic sheep may need correction to *P. cuniculi*.

In France in 1920, Henry (9) observed a high incidence of ear mites in horses, donkeys, and mules. He distinguished them from horse body mites on the basis of only two, rather than three, contiguous setal bases on the opisthosomal lobes of the adult male, and created the name *P. hippotis* Henry and Railliet, 1920. The validity of the distinguishing criterion was questioned by Hirst (11), who wondered if *P. hippotis* could be either *P. ovis* or *P. cuniculi*. In 1946, Lucas and Roberts (19) found ear mites in horses in Australia, where *P. ovis* had not been seen for over 50 years. They doubted the possibility of contact between horses and rabbits, and felt obliged to accept the host specificity attributed to psoroptic mites. *P. hippotis* was accepted, therefore, as valid. In the present study, a donkey was ascertained to be negative for ear mites, and then transfers were made of *P. cuniculi*. These proved successful. In the absence of any differentiating morphological character, together with the above experiment, it is proposed that *P. hippotis* be accepted as a synonym of *P. cuniculi*.

Canestrini (3) named *P. gazellae* from the ears of *Gazella*, but gave no description of the mite, or data on the geographical source, host species, or pathogenicity. In view of this dearth of information, and the non-specificity noted above for *P. cuniculi*, it would seem desirable, at least for the present, to regard *P. gazellae* as a synonym of *P. cuniculi*.

One other auricular species remains. It is *P. cervinus*, named by Ward in 1915 (44) from wild bighorn (*Ovis canadensis*) in Colorado. This species was

*Since the sheep shook their heads so vigorously following the first transfer, their ears were taped closed for 1 to 2 days following the second or third transfer to give the mites maximum opportunity for colonization.

created on (*a*) its geographical location, (*b*) its occurrence in the ears of a host indigenous to America, and (*c*) to a lesser degree on comparisons with Mégnin's (22) drawings of the body mites of horses. From examination of specimens, this writer believes that the morphological criteria used by Ward fall within the limits of individual variation for both kinds. There is, however, as clarified elsewhere, one, and sometimes a second, morphological feature that distinguish *P. cervinus* and *P. cuniculi*. These data suggest that there are two auricular psoroptic species, *P. cuniculi* in Old World hosts and *P. cervinus* in an American host. It seems likely also that the reports (12, 23) of *P. ovis* in the ears of bighorn may need correction to *P. cervinus*.

B. Body Mites

P. cervinus has also been reported in Wyoming (12, 25) from the bodies of wapiti (*Cervus canadensis*) which displayed lesions in winter similar to sheep scab. This writer has examined these mites and also believes them to be *P. cervinus*. This, however, is based on the length of the outer opisthosomal seta (Fig. 16), which clearly distinguishes *P. cervinus* from *P. cuniculi*, *P. equi*, and *P. natalensis*, but which is not always a distinguishing feature from *P. ovis* However, the length of this seta on many individuals from the wapiti always fell within the range established for material from bighorn. This range, as shown in Fig. 16, overlaps, but is different from that established in this paper for *P. ovis*. There is also some experimental data to distinguish *P. cervinus* from *P. ovis*. Later in this communication, *P. bovis* of domestic cattle is suggested as a synonym of *P. ovis*. At this point, it is significant that Murie (25) stated that a scabby wapiti was maintained with dairy cattle for a winter, but none showed any indication of having acquired mange. Hence, Murie's observation may be a biological feature distinguishing *P. ovis* from *P. cervinus*.

Apparently the ears of the infested wapiti were not examined. This writer had an opportunity to examine the ears of 40 wapiti from the herd at Elk Island Park, Alberta. Body mange has never been observed in this herd, and no mites occurred in their ears. Nor were psoroptic mites found in the ears of 7 moose (*Alces alces*) from the same park; or in over 200 reindeer (*Rangifer tarandus*) at Reindeer Depot, N.W.T.; or in 2 white-tailed deer (*Odocoileus virginianus*) from Quebec; or in 5 mule deer (*O. hemionus*) and 1 pronghorn (*Antilocapra americana*) from New Mexico. On the basis of the natural infestations, it seems possible that *P. cervinus*, like *P. cuniculi* and *Chorioptes texanus* (40), is essentially an ear mite that sometimes infests the body where it causes more pathology than when confined to the ears.

There are four recognized species or varieties of *Psoroptes* (*P. natalensis*, *P. ovis*, *P. bovis*, and *P. equi*) that infest the body of livestock. *P. natalensis* was described originally by Hirst (10) from specimens off domestic cattle in Natal. He also referred mites to this species that had been collected by Mégnin in 1885 (21) from a mangy Indian water buffalo (*Bubalus bubalis*) that had been some months in the Jardin de Plantes, Paris. The buffalo was originally from Cochin, China. Mégnin pointed out that this type of buffalo ("Arni à cornes en croissant") spent much time wallowing in water in its native

habitat, but that no water occurred in the beast's enclosure in the zoo. It is difficult to imagine how psoroptic mites—any more than chorioptic mites (39)—could survive more or less continuous soaking as they would in China, but one cannot state conclusively that the mites were acquired in France either. This, however, seems probable.

Besides these two reports, *P. natalensis* has been reported from cattle and zebu (*Bos indicus*) in South America (31). This writer has received psoroptic mites from cattle in New Zealand and from cattle and horses at Onderstepoort, South Africa, which are undoubtedly *P. natalensis*. These data extend both the known geographical distribution and host range of this species.

P. ovis has been reported as a natural infestation and producer of scab on domestic sheep and bighorn, both wild and captive (12, 34, 44). A review of the data in Seton (34) indicated that body mange may have been transferred from sheep to wild bighorn in the western United States from about 1885 onwards, after which large numbers of bighorn died with scab.* These data demonstrate that *P. ovis*, like *P. cuniculi*, *P. cervinus*, and *P. natalensis*, is not specific to a single host species.

It is stated repeatedly that *P. ovis* is distinct from *P. bovis* of domestic cattle. But published experimental evidence for this appears to be limited to the hundred-year-old report of Delafond and Bourguignon (in ref. 30) which stated that sheep psoroptic mange could not be communicated to the cow. Recently, Kemper and Peterson (14, 15) made valuable observations demonstrating transfer by direct bodily contact of psoroptic mange from sheep to one of three cows. Manual transfers followed from the positive cow to one of five other cattle. Incipient lesions appeared within 3 weeks and gradually spread to other body areas. Natural transfer occurred between the cattle and calves born subsequently into the herd. Transfers of mites from these cattle back to sheep were repeatedly successful in the early experiments, but appeared less successful 3 years later even though one of the three transfers succeeded at that time. Kemper and Peterson concluded with the suggestion that over the 3-year period *P. ovis* may have become gradually adjusted to existence on the new cattle host. Another explanation that fits the data just as well and which is in accord with data presented previously in this paper would be that individual sheep and cattle vary in their degree of susceptibility to infestation without any distinct adjustment by the mites to a particular host species. Natural infestations of body psoroptic mites may, therefore, be caused by the same mite on both hosts.

There is some historical evidence to suggest that *P. bovis* and *P. ovis* may be the same. In Australia, Canada, and New Zealand, sheep scab has been virtually eradicated. With its disappearance, bovine psoroptic mange has just about disappeared also. In the few sporadic cases that do occur occasionally, it would be of interest to ascertain if these mites, or *P. natalensis*, are

*Psoroptic mange has been suggested as having occurred on the bodies of bighorn in Canada (Stenton in ref. 5) but correspondence with appropriate people has revealed that mites were never seen and their possible confusion with normal molting ("cotting") may have resulted. Murie (24) did not observe the condition on Dall sheep (*Ovis dalli dalli*) in Alaska.

involved. These observations, together with the absence of any distinguishing morphological feature, indicate to this writer that *P. bovis* should be synonymized with *P. ovis*.

Data on psoroptic mites from horses are less convincing. Previous workers (see ref. 26) have shown that the bodies of some, but not all, horses have been infested temporarily, together with some subsequent mange, by the ear mite *P. cuniculi*. Gerlach (8) and Delafond (in ref. 30) were unsuccessful in transmitting psoroptic mange from the bodies of horses to cattle, but Gohier and Carrère (in ref. 30) believed that they had succeeded. Gohier and Müller (in ref. 26), however, observed that mites from cattle did not survive on the horse. There is no recent experimental evidence. This writer, as shown previously, has distinguished two kinds of psoroptic mites from horses on the basis of length of the outer opisthosomal seta on the adult male. One has been designated as the true *P. equi*. The other may be simply *P. ovis* on another host. The horse then becomes the host of four psoroptic mites: *P. ovis*, *P. equi*, and *P. natalensis* on the body, and *P. cuniculi* in the ears and occasionally on the body.

In summary, some types of psoroptic mites have been shown to be nonspecific, and host susceptibility appears to be related to the individual rather than the species to which the individual belongs. Where no biological or morphological differences exist between the types of *Psoroptes*, synonymy has been suggested. All species recognized herein can be distinguished on a population basis when both biological and morphological criteria are used.

Differential Diagnosis

The following key may be used to separate the species in *Psoroptes*. When a measurement is being made of the length of a seta, it is imperative to follow it to its end and avoid the use of any that are broken. The key will be most reliable, and perhaps only so, when a number of mites are available for study.

KEY TO THE SPECIES OF *Psoroptes* (ADULT MALES)

a.	Ear mite (essentially)	*b*
	Body mite	*c*
b.	Adult male with outer opisthosomal seta 64–164 μ	*P. cuniculi*
	Adult male with outer opisthosomal seta 145–354 μ	*P. cervinus*
c.	Adult male with some opisthosomal setae spatulate	*P. natalensis*
	Adult male with all opisthosomal setae threadlike	*d*
d.	Adult male with outer opisthosomal seta about 333 μ	*P. equi*
	Adult male with outer opisthosomal seta 74–258 μ	*P. ovis*
	Adult male with outer opisthosomal seta 145–354 μ and in America	*P. cervinus*

III. Grouping of the Genera and Species within Psoroptidae

Yunker (45) illustrated a possible phylogenetic relationship of Psoroptidae with the other families in the supercohort Acaridiae. The aim of the current discussion is to construct a dendrogram of the family Psoroptidae based on both biological and morphological data. Figure 17 shows that Psoroptidae divides into two natural groups herein designated Psoroptinae subfam. n.

with *Psoroptes* and Chorioptinae subfam. n. with *Chorioptes*, *Caparinia*, and *Otodectes*. The characteristics separating Psoroptinae and Chorioptinae are:

1. Their comparative size—*Psoroptes* is structurally larger than *Chorioptes*, *Caparinia*, and *Otodectes*, which are all about the same size.

2. The Psoroptinae have long, jointed, pedunculated caruncles on their legs, while those on the Chorioptinae are short and unjointed.

3. Their differences in feeding. The chelicerae of the Chorioptinae are strongly chelate and they feed by chewing essentially on, if not exclusively on, epidermic debris. For all intents and purposes, they are saprophytic. The Psoroptinae, on the other hand, have elongate, chelate chelicerae that might best be described as piercing and chewing. They cause direct damage to the skin and are truly parasitic.

Perhaps the early type of psoroptid mite was saprophytic on dead host tissue and developed from a similar free-living habitat. Then the mites would not be confined to a particular site on the host and could have occurred in both the ears and on the body. This would make parallel evolution possible in Psoroptinae and both lines of Chorioptinae (see Fig. 17) resulting in today's ear-infesting and body-infesting species in each group. This seems possible since the ear mites *C. texanus*, *P. cuniculi*, *P. cervinus*, and *O. cynotis* not infrequently live on the body as well as in the ear, and must wander onto the body from the ears to facilitate transfer between hosts.

In the subfamily Psoroptinae, *P. natalensis* is placed as the earliest offshoot from the main line and closest within the group to *Chorioptes* since the adult males of both *P. natalensis* and *Chorioptes* spp. have spatulate setae on their opisthosomal lobes and the important host for *P. natalensis* and *C. bovis* appears to be the domestic cow. In the absence of indisputable morphological distinctions, it is difficult to know whether the four remaining types in *Psoroptes* should be considered nascent species or be given specific rank. The latter has been chosen somewhat arbitrarily. There is little doubt that the ear mites (*P. cuniculi* and *P. cervinus*) are completely distinct from the body mites (*P. ovis* and *P. equi*), but whether each pair is completely subdivisible is questionable. *P. ovis* and *P. equi*, being on farm animals, have not been separated geographically in historic times. The only pertinent paleontological evidence pertains to bighorn and wapiti, the hosts of *P. cervinus*, which suggests that *P. cervinus* has been separated from the Old World types for at least 30,000 years. Scott (33) writes that the bighorn and wapiti occurred in North American Pleistocene deposits, but no archaic types have been found in any deposits of an earlier date. It was deduced from this that these two mammals were Asiatic immigrants that arrived in the Western Hemisphere during the Pleistocene over the land bridge that joined Asia and America from the middle Miocene to the late Pleistocene. The retreat of the last ice sheet began about 30,000 years ago, so the bighorn and wapiti, and with them *P. cervinus* or its prototype, have been separated from the Asiatic types for that period, but not necessarily longer. Clearly, there is limited evidence for separating *P. cuniculi* from *P. cervinus* and also *P. ovis* from *P. equi*, and the

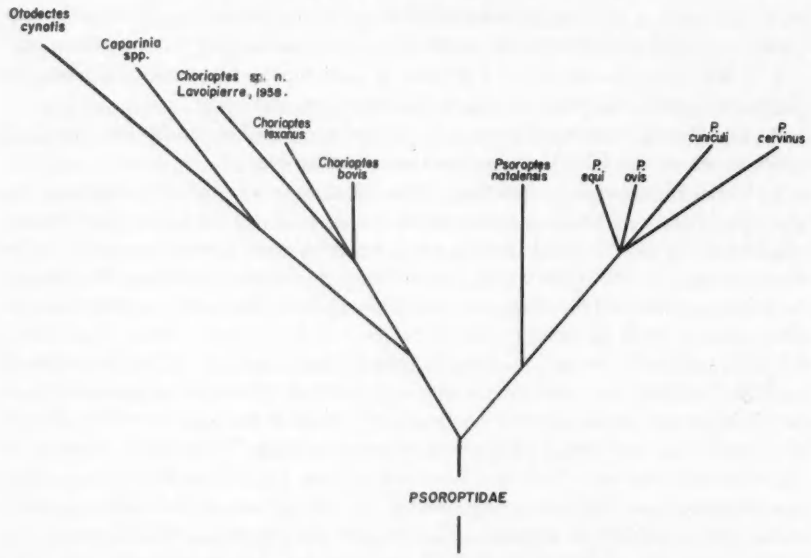


FIG. 17. Phylogenetic relationships within Psoroptidae.

current data might only justify taxonomic rank below that of species, but for want of further research, specific rank for each has been chosen in this paper.

The ear mites *P. cuniculi*, *P. cervinus*, and *C. texanus*, but not *Chorioptes* sp. n. Lavoipierre, 1958 (17), are placed approximately at the same phylogenetic level in Fig. 17 for the following reasons. Each shows sexual dimorphism in the nymphal stages. All can, and sometimes do, infest the body where they are usually pathogenic. The adult male is the only stage that morphologically separates *P. cuniculi* from *P. cervinus*, and *C. texanus* from *C. bovis*. However, *Chorioptes* sp. n. Lavoipierre, 1958 from the ears of the red-flanked duiker (*Cephalophus rufilatus*) in the British Cameroons (17) is placed at a higher level of specialization than the above because all stages of this newly discovered parasite are morphologically distinct from those in the old species of *Chorioptes*. In the genus *Chorioptes*, the three species appear to have evolved in hosts in three climatic zones: *C. bovis* on Bovidae and Equidae in a temperate climate, *C. texanus* in the ears of reindeer and body of goats in an arctic–alpine–temperate climate, and *Chorioptes* sp. n. Lavoipierre, 1958 in the ears of an antelope in a tropical climate.

Within the Chorioptinae subdivision, the genus *Chorioptes* is placed closer to the Psoroptinae than is *Caparinia* or *Otodectes* since *Chorioptes*, like *Psoroptes*, parasitizes herbivores while *Caparinia* and *Otodectes* are natural parasites of carnivores and an insectivore. Also, the two former genera have the same number of stages in their life cycle while *Otodectes*, and probably *Caparinia*, have a reduced number. Although *Otodectes* and *Caparinia* occur on carnivores and an insectivore, they may have been derived from a primitive type on a

herbivore. This was suggested by discovery (41) of *Otodectes cynotis* on the body of a captive white-tailed deer (*Odocoileus virginianus*) suggesting that herbivores may become hosts under unusual or artificial conditions. In both *Otodectes* and *Caparinia* the length of the fourth leg is much reduced in the protonymph, and it completely disappears in the deutonymph of *Otodectes* but persists in the deutonymph of *Caparinia*. This places *Caparinia* between *Otodectes* and *Chorioptes*. The adult males in both genera stand a 50% chance of attachment with a deutonymph which itself emerges into an adult male, demonstrating specialization that has led to some reproductive failure, or "biological waste". Because of these data, the *Otodectes–Caparinia* line is considered the most highly specialized in the family. Clearly, Psoroptidae has two main divisions (Psoroptinae and Chorioptinae), one of which subdivides to include parasites of herbivores on the one hand and more specialized parasites of carnivores and an insectivore on the other.

Summary

The life history of *P. cuniculi* is described, and the stages in a single cycle include the egg, larva, protonymph, deutonymph, and adult. The egg and larva show no sexual dimorphism, but the sex of the remaining stages is ascertainable. Other types in the genus have the same life cycle. Host specificity is disproved, and the same mammal is shown to be on occasion the host of more than one kind of psoroptic mite. Auricular mites sometimes occur on the body, but there is no evidence that body mites can live in the ears. Ear mites from the rabbit (*P. cuniculi*), goat (*P. equi* var. *caprae*), horse, donkey, mule (*P. hippotis*), domestic sheep (*P. ovis* when in the ears only), and *Gazella* (*P. gazellae*) are declared synonymous. *P. cuniculi* has priority. One other auricular mite, namely *P. cervinus* (emended from *P. cervinae*) in American hosts, remains valid. The host range of the body mite *P. ovis* is extended to include not only domestic sheep and bighorn, but also domestic cattle and horses. *P. bovis*, but not *P. equi* or *P. natalensis*, is suggested as a synonym of *P. ovis*. The species recognized herein can be distinguished on a population basis by using both morphological and biological criteria. Their names, hosts, and geographical distributions are:

P. cuniculi (Delafond, 1859) Canestrini and Kramer, 1899—ear mite of the rabbit, goat, sheep, horse, donkey, mule, and possibly *Gazella*. Also a temporary body mite of horses without occurring in the ears. Cosmopolitan.

P. cervinus Ward, 1915—ear mite of the bighorn and body mite of wapiti. Western United States.

P. natalensis Hirst, 1919—body mite of domestic cattle, zebu, Indian water buffalo, and horse. South Africa, Uruguay, Brazil, New Zealand, and probably France.

P. equi (Hering, 1838) Gervais, 1841—body mite of the horse, donkey (?), and mule (?). England only sure country.

P. ovis (Hering, 1838) Gervais, 1841—body mite of domestic sheep, bighorn, cattle, horse, donkey (?), and mule (?). Cosmopolitan.

The phylogenetic relationship of these species with each other and with the species in *Chorioptes*, *Caparinia*, and *Otodectes* is discussed, together with creation of Psoroptinae subfam. n. and Chorioptinae subfam. n. for two distinct lines of development.

Acknowledgments

Particular thanks is extended to Dr. P. J. G. Plummer and Dr. J. W. Pullin of the Health of Animals Division, Canada Department of Agriculture, and to the Director, Institute of Parasitology, for their co-operation throughout the period of study. Messrs. D. V. Conlon, J. Mackie, and W. Goulet provided reliable assistance in the laboratory and in the maintenance of the experimental animals. Psoroptic mites from hosts in various world centers, or assistance in other ways, were kindly provided by Dr. W. N. Beesley, Ministry of Agriculture, Fisheries and Food, Weybridge; Dr. E. M. DuPorte, Macdonald College, McGill University; Dr. R. DuToit, Division of Veterinary Services, Union of South Africa; Dr. G. Owen Evans, British Museum of Natural History, London; Dr. R. F. Honess, Wyoming Game and Fish Commission, Cheyenne; Dr. A. A. Kingscote, Department of Parasitology, Ontario Veterinary College; Dr. G. Lubinsky, Institute of Parasitology, Macdonald College; Dr. H. O. Peterson, United States Department of Agriculture, Albuquerque, New Mexico; Dr. R. F. Riek, C.S.I.R.O., Yeerongpilly, Australia; Dr. G. A. Shad, United States Department of Agriculture, State College, New Mexico; Dr. R. W. Strandtmann, Texas Technological College, Lubbock; Dr. G. W. Wharton, Department of Zoology, University of Maryland; and Dr. L. K. Whitten, Wallaceville Animal Research Station, New Zealand.

References

1. Babcock, O. G. and Black, W. L. The common sheep-scab mite and its control. Bull. Texas Agr. Exptl. Sta. No. 479 (1933).
2. Buxton, P. A. The capitulum of *Psoroptes*. Parasitology, **12**, 334–336 (1920).
3. Canestrini, G. Prospetto dell'acarofauna Italiana. Part VI*a*. 1894.
4. Canestrini, G. and Kramer, P. Demodicidae and Sarcoptidae *in* Das Tierreich, Lief 7. *Edited by* Verlag von R. Friedländer und Sohn, Berlin. 1899.
5. Cowan, I. McT. Parasites and injuries of game animals in the Rocky Mountain National Parks. (Mimeographed.) 1942–1944.
6. Downing, W. The life history of *Psoroptes communis* var. *ovis* with particular reference to latent or suppressed scab. J. Comp. Path. Therap. **49**, 163–180 and 183–209 (1936).
7. Gedoelst, L. Le parasite de l'otacariase des chèvres du Congo. Arch. Schiffs- und Tropen-Hyg. **13**, 150–152 (1909).
8. Gerlach, A. C. Krätze und Räude, entomologisch und klinisch bearbeitet. August Hirschwald, Berlin. 1857.
9. Henry, A. Les *Psoroptes* de l'oreille des equidés (*Psoroptes hippotis* Railliet et Henry nov. sp.). Bull. Soc. Cent. Méd. Vét. **73**, 41–43 (1920).
10. Hirst, S. On two new parasitic mites (*Mycoptes hintoni* and *Psoroptes natalensis*). Ann. and Mag. Nat. Hist. **3**, Ser. 9, 524 (1919).
11. Hirst, S. Mites injurious to domestic animals (with an appendix on the acarine disease of hive bees). Brit. Museum (Nat. Hist.), Econ. Ser. No. 13. 1922.
12. Honess, R. F. and Winter, K. B. Diseases of wildlife in Wyoming. Wyoming Game and Fish Commission. 1956.
13. Imes, M. Cattle scab. U.S. Dept. Agr. Farmers' Bull. No. 713, Washington. 1927.
14. Kemper, H. E. and Peterson, H. O. Transmission of psoroptic sheep scab to cattle. (Mimeographed.) 1953.

15. KEMPER, H. E. and PETERSON, H. O. Scabies in sheep and goats, *in* Animal diseases. Yearbook Agr., U.S. Dept. Agr., Washington. 1956.
16. KEMPER, H. E. and ROBERTS, I. H. Eradication of sheep scabies. Proc. U.S. Livestock Sanitary Assoc. **54**, 247–253 (1950).
17. LAVOIPIERRE, M. M. J. Personal communications *and* Ann. Trop. Med. Parasitol. (1958). (In press.)
18. LUCAS, G. C. *Psoroptes ovis* (Hering) Gervais. Contribucion al estudio de su ciclo evolutivo. Rev. med. vet. **28**, 406–426 (1942).
19. LUCAS, K. M. and ROBERTS, F. H. S. Psoroptic otacariasis of the horse. Aust. Vet. J. **22**, 186 (1946).
20. LUCET, A. Une familie de lapins réfractaire à la gale auriculaire. Rec. méd. vét. **67**, 96–97 (1890).
21. MÉGNIN, J. P. Constatation de l'existence de la gale chez le buffle de l'Inde. Compt. rend. soc. biol. **37**, 705–706 (1885).
22. MÉGNIN, J. P. Monographie de la tribu des sarcoptides psoriques qui comprend tous les acariens de la gale de l'homme et des animaux. Deuxième Partie. Histoire naturelle des acariens qui constituent la tribu des sarcoptides psoriques. Rev. Mag. Zool. Pur. Appl. **40**, 82–173 (1877).
23. MILLS, H. B. A preliminary study of the bighorn of Yellowstone National Park. J. Mammal. **18**, 205–212 (1937).
24. MURIE, A. The wolves of Mount McKinley. Fauna of the National Parks of the United States. Fauna Series No. 5, Washington. 1944.
25. MURIE, O. J. The elk of North America. Stackpole Co. *and* the Wildlife Management Institute, Harrisburg *and* Washington. 1951.
26. NEUMANN, L. G. A treatise on the parasites and parasitic diseases of the domesticated animals. *Translated by* G. Fleming. William R. Jenkins, New York. 1892.
27. NEVEU-LEMAIRE, M. Traité d'entomologie médicale et vétérinaire. *Edited by* Vigot Frères, Paris. 1938.
28. PALIMPSESTOV, M. A. [Differentiating characters of the larvae of mange mites in the genus *Psoroptes* Gervais, 1841.] (In Russian.) Veterinariya, **21**, 23–24 (1944).
29. PRISELKOVA, D. O. [Morphology, biology and ecology of mange mites from horses.] (In Russian.) Rept. First Soviet Conf. Vet. Derm. 59–63 (1954).
30. RAILLIET, A. Traité de zoologie médicale et agricole. Part I. Asselin et Houzeau, Paris. 1893.
31. ROCHA, U. F., MALHEIRO, D. M., and CUNHA, J. M. V. Sarna psoroptica em bovinos das raças Européias e Indianas, no estado de São Paulo, Brazil. *Psoroptes natalensis* Hirst, 1919 sinônimo de *P. equi* var. *bovis* (Gerlach, 1857). Rev. fac. med. vet. **4**, 537–544 (1952).
32. ROE, F. G. The North American buffalo—a critical study of the species in its wild state. Univ. of Toronto Press, Toronto. 1951.
33. SCOTT, W. B. A history of land mammals in the western hemisphere. Rev. Ed. The Macmillan Co., New York. 1937.
34. SETON, E. T. Lives of game animals, Vol. III, Part II, hoofed animals. Doubleday, Doran and Co. Inc., New York. 1929.
35. SHILSTON, A. W. Sheep scab. Observations on the life-history of *Psoroptes communis* var. *ovis*, and some points connected with epizootiology of the disease in South Africa. Third and Fourth Rept. Director Vet. Research, Dept. Agr., U.S.Af. 1915.
36. STOCKMAN, S. and BERRY, A. H. The *Psoroptes communis ovis;* some observations on ova and ovipositing. J. Comp. Pathol. Therap. **26**, 45–50 (1913).
37. SWEATMAN, G. K. Seasonal variations in the sites of infestation of *Chorioptes bovis*, a parasitic mite of cattle, with observations on the associated dermatitis. Can. J. Comp. Med. Vet. Sci. **20**, 321–336 (1956).
38. SWEATMAN, G. K. Life history, non-specificity, and revision of the genus *Chorioptes*, a parasitic mite of herbivores. Can. J. Zool. **35**, 641–689 (1957).
39. SWEATMAN, G. K. On the population reduction of chorioptic mange mites on cattle in summer. Can. J. Zool. **36**, 391–397 (1958).
40. SWEATMAN, G. K. Redescription of *Chorioptes texanus*, a parasitic mite from the ears of reindeer in the Canadian arctic. Can. J. Zool. **36**, 525–528 (1958).
41. SWEATMAN, G. K. Biology of *Otodectes cynotis*, the ear canker mite of carnivores. Can. J. Zool. **36**, 849–862 (1958).
42. VITZTHUM, H. G. Ordnung der Arachnida: Acari = Milben *in* Handbuch der Zoologie III, 1–160. Walter de Gruyter & Co., Berlin and Leipzig. 1931.
43. VITZTHUM, H. G. Acarina *in*, Klassen und Ordnungen des Tierreichs, 5. Band: Arthropoda, IV Abteilung: Arachnoidea, 5. Buch. *by* H. G. Bronns. Becker & Erler Kom.-Ges., Leipzig. 1943.
44. WARD, H. B. Otacariasis in the bighorn. J. Parasitol. **1**, 121–127 (1915).
45. YUNKER, C. E. A proposed classification of the Acaridiae (Acarina: Sarcoptiformes). Proc. Helminthol. Soc. Wash. **22**, 98–105 (1955).

A CHEMICALLY DEFINED DIET AND AXENIC REARING METHOD FOR LARVAE OF THE SEED-CORN MAGGOT, HYLEMYA CILICRURA (ROND.) (DIPTERA : ANTHOMYIIDAE)[1]

W. G. FRIEND,[2] E. H. SALKELD,[2] AND R. J. MCCLANAHAN[3]

Abstract

Larvae of the seed-corn maggot, *Hylemya cilicrura* (Rond.), were reared on two chemically defined diets under axenic conditions. The diets consisted of 19 *l*-amino acids, 9 water-soluble vitamins, coenzyme A, inosine, thymine, ribonucleic acid, glucose, a salt mixture, water, and agar. They contained no plant extracts or unknown growth factors. Eighty-three per cent of the 29 larvae reared axenically on the better diet became adults. The larvae developed approximately half as fast as those reared on pea seedlings.

Introduction

Many of the nutritional requirements of larvae of the onion maggot, *Hylemya antiqua* (Meig.), have been determined through the use of various chemically defined diets and axenic rearing techniques (2–5). To extend these studies to other species of *Hylemya* various chemically defined diets were tested for the seed-corn maggot, *H. cilicrura* (Rond.), and the cabbage maggot, *H. brassicae* (Bouché), all attempts to rear *H. brassicae* larvae on the diets having failed (5). This paper describes two chemically defined diets differing in their concentration of nutrients and an axenic rearing technique used to rear *H. cilicrura* from egg to adult.

Methods

The diets used were similar to the chemically defined diet developed for larvae of the onion maggot (3). A preliminary experiment (5) had indicated that larvae of *H. cilicrura* developed better on a diet in which the concentrations of nutrients were only 50 to 75% of those for the onion maggot diet. Two diets (Tables I–III) were formulated in which the concentrations of nutrients were 66.6% and 50% of those for the onion maggot diet. The diets were as nearly chemically defined as possible although the ribonucleic acid and the agar may contain unknown materials in trace amounts; no plant extract or other crude material was used.

Preparation of Diets

A stock diet was prepared in which the concentrations of nutrients were twice those needed for the low-level diet (Tables I–III). A 333.2-ml aliquot of the stock diet was diluted to 500 ml to form the high-level diet, and 250 ml was diluted to 500 ml for the low-level diet.

[1]Manuscript received September 4, 1958.
Contribution No. 3846, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Crop Insect Section, Entomology Laboratory, Ottawa, Ontario.
[3]Entomology Laboratory, Chatham, Ontario.

TABLE I

COMPOSITIONS OF THE DIETS IN AMOUNTS PER MILLILITER

Constituents	High-level diet, mg	Low-level diet, mg	High-level diet, μg	Low-level diet, μg
Agar	30.0	30.0		
Ribonucleic acid	0.67	0.50		
Inosine			20.0	15.0
Thymine			2.7	2.0
Cholesterol			67.0	50.0
Dextrose	10.0	7.5		
Mineral mixture U.S.P. XIII, No. 2*	1.3	1.0		
Amino acid mixture (see Table II)	14.69	11.30		
Vitamin mixture (see Table III)			40.70	30.36

*Calcium biphosphate, 13.58%; calcium lactate, 32.7%; ferric citrate, 2.97%; magnesium sulphate, 13.70%; potassium phosphate (dibasic), 23.98%; sodium biphosphate, 8.82%; sodium chloride, 4.35%.

TABLE II

AMOUNTS MG/ML OF VARIOUS AMINO ACIDS IN DIETS

Amino acid	High-level diet	Low-level diet
l-Alanine*	0.73	0.55
l-Arginine (free base)*	0.53	0.40
l-Aspartic acid†	0.81	0.61
l-Cysteine (free base)*	0.32	0.24
l-Glutamic acid*	1.48	1.11
Glycine*	1.17	0.88
l-Histidine (free base)*	0.32	0.24
l-Hydroxyproline†	0.25	0.19
l-Isoleucine*	0.84	0.63
l-Leucine†	1.57	1.18
l-Lysine HCl†	0.89	0.67
l-Methionine†	0.23	0.17
l-Phenylalanine†	0.68	0.51
l-Proline†	1.12	0.84
l-Serine*	0.59	0.44
l-Threonine*	0.25	0.19
l-Tryptophan*	1.17	0.88
l-Tyrosine*	0.83	0.62
l-Valine*	0.91	0.68
Total	14.69	11.03

*Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.
†Mann Research Laboratories Inc., New York, N.Y., U.S.A.

The stock diet was prepared in the following way: The amino acids listed in Table II, excepting aspartic acid, glutamic acid, tryptophan, and tyrosine, were weighed in amounts required to make 1 liter of diet containing twice the concentration of the low-level diet.

Stock solutions of the four least soluble amino acids were formulated and the correct amounts for the stock diet were mixed as follows: *l*-aspartic acid (12.2 mg per ml solution in water), 80 ml; *l*-glutamic acid (22.1 mg per ml solution in water), 100 ml; *l*-tryptophan (17.5 mg per ml solution in water), 70 ml; and *l*-tyrosine (12.4 mg per ml solution in 3% HCl), 50 ml.

The 300 ml of amino acid solution and 200 ml of water were then rapidly poured over the dry amino acids in a 2000-ml beaker. The resulting mixture was stirred violently until it was dissolved.

Six grams of U.S.P. XIII No. 2 salt mixture was mixed with 300 ml of water; when the insoluble fraction had settled, 100 ml of the supernatant was decanted into the amino acid mixture.

A stock solution of inosine and thymine was made by adding 300 ml of hot water to 90 mg of inosine and 12 mg of thymine, and warming them in a water bath for 1 hour. One hundred milliliters of this solution was then added to the diet mixture.

The 1 g of ribonucleic acid in the diet was treated carefully to prevent "burning" through exposure to too high a pH. Seventy milliliters of water was poured over the dry polynucleotide, and the mixture was stirred rapidly to minimize clumping; 3 ml of 2*N* NaOH was then added dropwise. When the ribonucleic acid was completely dissolved, water was added to bring the volume up to 100 ml. Experience had shown that adding the ribonucleic acid solution directly to the amino acid mixture caused the ribonucleic acid to precipitate out of solution. The addition of 5 ml of 3*N* KOH and 5 ml of 3*N* NaOH to the amino acid mixture before addition of the 100 ml of ribonucleic acid solution prevented precipitation.

Stock solutions of the vitamins listed in Table III were prepared in the following concentrations: biotin, 2.2 μg per ml, in water; B_{12}, 4.5 μg per ml, in water; calcium pantothenate, 700 μg per ml, in water; choline chloride, 2300 μg per ml, in water; coenzyme A, 170 μg per ml, in water; folic acid, 700 μg per ml, in 20% ethyl alcohol; nicotinic acid, 1200 μg per ml, in water; pyridoxine HCl, 3400 μg per ml, in water; riboflavin, 135 μg per ml, in warm 0.02 *N* acetic acid; thiamine HCl, 170 μg per ml, in water; and *dl*-6-thioctic acid, 560 μg per ml, in 4% ethyl alcohol.

A vitamin mixture consisting of 20 ml of the riboflavin solution and 10 ml of each of the other stock vitamin solutions was prepared; the 120 ml of vitamin mixture was added to the diet, bringing the volume of the diet mixture up to 930 ml.

TABLE III

AMOUNTS μG/ML OF VARIOUS VITAMINS IN DIETS

Vitamin	High-level diet	Low-level diet
Biotin (aqueous solution)	0.013	0.01
B_{12}	0.033	0.025
Calcium pantothenate	0.46	0.35
Choline chloride	0.16	0.12
Coenzyme A*	1.13	0.85
Folic acid	4.60	3.50
Nicotinic acid	8.00	6.0
Pyridoxine HCl	23.00	17.0
Riboflavin	1.80	1.35
Thiamine HCl	1.10	0.85
dl-6-Thioctic acid*	0.40	0.30
Total	40.696	30.355

*Not yet classed as a vitamin but suspected of having vitamin activity in *H. ciliatura*.

A stock solution of cholesterol was made by dissolving 500 mg of cholesterol in 20 ml of hot 95% ethyl alcohol. This was then pipetted into 100 ml of cold distilled water to which 0.3 ml of polyoxyethylene sorbitan monooleate (Tween 80, Brickman and Co., Montreal, Canada) had been added. This gave a milky suspension, which was reduced to 100 ml by evaporation under vacuum to remove as much of the alcohol as possible. Twenty milliliters of this suspension was added to the diet mixture, dropwise with stirring to disperse the cholesterol particles. This brought the volume of the diet mixture up to 950 ml.

Fifteen grams of glucose was added to the diet mixture, which was then warmed on a water bath and stirred until an opalescent suspension resulted. The pH of the diet mixture was then adjusted to 6.1 by adding 6.3 ml of a mixture of 50% 3*N* KOH and 50% 3*N* NaOH. The volume of the diet mixture was then brought up to 1000 ml by adding distilled water.

The aliquots needed to provide the necessary nutrients for the high- and low-level diets were measured out; each was diluted to 500 ml and 10 g of agar was added to each. The agar was melted by heating the diets in an autoclave. The diets were then dispensed into diet tubes, 1.5 to 2 ml of diet being placed in each tube. The diet tubes were 1-dram screw-capped vials with the cardboard liners removed from the bakelite caps so that the caps could be thoroughly cleaned. After being charged with diet, the tubes were marked, loosely capped, and autoclaved for 15 minutes at 15 lb pressure. The diet tubes were then removed from the autoclave and stacked so that the agar formed slants in the tubes.

Two hundred and thirty-three tubes of the high-level diet and 203 of the low-level diet were compounded in this fashion.

Preparation of Axenic Eggs

Eggs were obtained from a culture of the seed-corn maggot maintained at the Chatham laboratory, the insect being reared by the method of McClanahan and Miller (7). The eggs were immersed in a 20% formalin solution for 1 hour and then washed in sterile water. Eggs less than 24 hours old were used. After being washed, the eggs were transferred to the surface of the diet in the diet tubes by means of a platinum loop, one egg being placed in each tube. The loop and the mouth of the diet tube were flamed at each transfer to maintain axenic conditions in the tube.

Assay Procedure

The diet tubes were incubated at $25 \pm 1°$ C throughout the assay period. Each day the tubes were inspected with a dissecting microscope. The larvae could be clearly seen tunnelling in the transparent diets. The anatomical changes in the mouth hooks (1) from instar to instar were used to measure larval development. Records were kept of the development of each larva.

As soon as any sign of contamination by microorganisms was noticed in any of the diet tubes, the contaminated tubes were discarded. When pupation occurred in a diet tube a portion of the diet was smeared on a slide and stained with crystal violet, and 20 fields were inspected at 800× magnification. If any bacterial cells were found the results from that tube were discounted.

Pupation occurred at the surface of the diet. Because some of the flies emerging from the puparia became stuck in the diet it would have been better if the puparia had been removed from the diet tubes and placed in moist sand or moss.

Results and Discussion

Table IV shows that there was little difference in the development rates of larvae grown on the two diets. The period from hatching to pupation, 12 to 19 days and 12 to 27 days on the high- and low-level diets, respectively, was considerably longer than the 8 to 10 days noted by McClanahan and Miller for the seed-corn maggot when reared on pea seedlings under laboratory conditions at 80° F (7). This increase in development time may be partially caused by the lower incubation temperature, 77° F, used during the larval period in the writers' experiment. It is noteworthy, however, that the average pupal period for the insects reared on the chemical diets was about 9 days compared with 5 days for pupae from larvae reared on pea seedlings (7) even though the incubation temperatures during the pupal stage were higher, 77° F, in the former case than in the latter, 65–75° F (7).

The adults formed from larvae reared on both chemical diets appeared normal but no attempt was made to determine whether they could lay viable eggs.

The scope of this study was limited by the large number of nonviable eggs. Of 436 eggs used, 61% did not hatch. This corresponds closely to the 60% mortality in the egg and first-instar stages noted by McClanahan and Miller (7).

TABLE IV

DEVELOPMENT OF *H. cilicrura* ON TWO CHEMICAL DIETS

	High-level diet	Low-level diet
Number of insects on diet*	46	29
Average time, in days, from hatching to:		
second instar	3.3 (2–4)	3.7 (3–5)
third instar	6.1 (4–8)	6.1 (5–8)
pupa	15.2 (12–19)	15.4 (12–27)
adult	24.5 (22–39)	24.5 (22–30)
Percentage of total number reaching:		
second instar	100	100
third instar	100	97
pupa	85	90
adult	76 (63 ♂, 37 ♀)	83 (50 ♂, 50 ♀)

*Only aseptic insects in normal diet tubes considered.

Contamination of the diet tubes by microorganisms was high. Of 233 eggs placed on the high-level diet, 52% became contaminated as did 56% of the 203 eggs placed on the low-level diet. Diet tubes showing any sign of contamination were discarded.

Either of the two diets used would provide a satisfactory basal diet for a study of the nutritional requirements of the seed-corn maggot. Because of the high percentage of nonviable eggs, some method of placing axenic, newly hatched larvae on the diet in the tubes, such as that described by House (6) for larvae of *Pseudosarcophaga affinis* (Fall.), would be of advantage.

References

1. BROOKS, A.R. Identification of the root maggots (Diptera:Anthomyiidae) attacking cruciferous garden crops in Canada, with notes on biology and control. Can. Entomologist, **83**, 109–120 (1951).
2. FRIEND, W. G. and PATTON, R. L. Studies on vitamin requirements of larvae of the onion maggot, *Hylemya antiqua* (Meig.), under aseptic conditions. Can. J. Zool. **34**, 152–162 (1956).
3. FRIEND, W. G., BACKS, R. H., and CASS, L. M. Studies on amino acid requirements of larvae of the onion maggot, *Hylemya antiqua* (Meig.), under aseptic conditions. Can. J. Zool. **35**, 535–543 (1957).
4. FRIEND, W. G. The nutrition of phytophagous insects with special reference to *Hylemya antiqua* (Meig.). Proc. 10th Intern. Congr. Entomol., Montreal. (In press.)
5. FRIEND, W. G. and SALKELD, E. H. Nutrition of onion maggots, larvae of *Hylemya antiqua* (Meig.), with reference to other members of the genus *Hylemya*. Ann. New York Acad. Sci. (In press.)
6. HOUSE, H. L. Nutritional studies with *Pseudosarcophaga affinis* (Fall.), a dipterous parasite of the spruce budworm, *Choristoneura fumiferana* (Clem.). I. A chemically defined medium and aseptic culture technique. Can. J. Zool. **32**, 331–341 (1954).
7. MCCLANAHAN, R. J. and MILLER, L. A. Laboratory rearing of the seed-corn maggot, *Hylemya cilicrura* (Rond.) (Diptera: Anthomyiidae). Can. Entomologist, **90**, 372–374 (1958).

OPHRYOSCOLECIDAE (CILIATA : ENTODINIOMORPHIDA) OF THE REINDEER (RANGIFER TARANDUS L.) FROM THE CANADIAN ARCTIC

II. DIPLODINIINAE[1]

G. LUBINSKY

Abstract

This paper is concerned with the Diplodiniinae of 14 reindeer from the Canadian Arctic. One new species, *Polyplastron arcticum* n.sp., is described, and eight other species of Diplodiniinae redescribed. A key to the species of Diplodiniinae of reindeer has been compiled.

Introduction

This paper is concerned with Diplodiniinae collected from reindeer in Aklavik by Dr. L. K. Whitten. The terminology used in describing these ciliates was discussed in the first paper of this series. The methods of examination described in the previous paper were employed (11). In addition, lactic acid, to which 1/10th of its volume of 10% alcoholic solution of iodine was added, was used for clearing the ciliates and staining their skeletons. Thick films, cleared and mounted in this medium, remain adequately stained for several months. Nine species of Diplodiniinae were found in the material.

Description of Species

1. *Diplodinium rangiferi* Dogiel, 1925 (s. str.) (Figs. 1–12), (3:49–51, Fig. 9), (8:84)

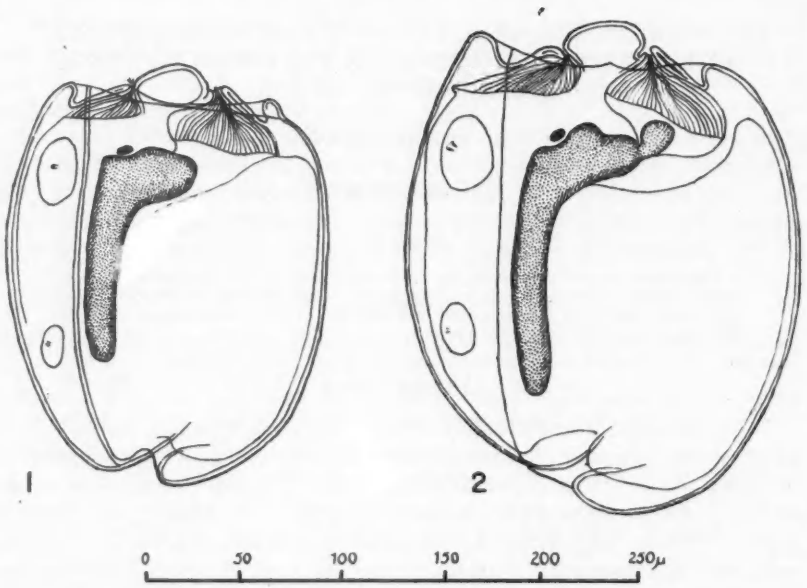
Syn.: *Anoplodinium rangiferi major* (4:99–100, Fig. 53), (6:147)

Body a wide ellipse with truncated anterior end, 201 (156–258) μ long, 166 (144–204) μ wide, length to width ratio 1.2 (1.08–1.37).

Oral area slanted to right at an angle of about 10–20°. Metoral membranelle zone nearly level with oral zone. Operculum small. Right and left body sides nearly equally convex. Right caudal lobe rounded, inconspicuous, 9(3–21) μ long; left lateral furrow on the upper body side between nuclear apparatus and contractile vacuoles. It begins at the anterior margin of outer lip of metoral membranelle zone, and on reaching the posterior end of body, turns nearly at right angles towards right caudal lobe to form the groove in which anus opens.

Endoplasmic sac very large, closely applied to body walls, not penetrating into operculum. Rectum comparatively narrow, opening into the wide, funnel-shaped caudal end of left lateral furrow. Contractile vacuoles between this furrow and left body margin.

[1]Manuscript received June 15, 1958.
Contribution from the Institute of Parasitology, McGill University, Macdonald College, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.



FIGS. 1 and 2. *Diplodinium rangiferi* Dogiel, 1925, from the upper ("right") side.

Macronucleus large, boomerang to hook-shaped, its longitudinal portion about half as long as body, its transverse portion about one third of body width, varying in shape from a rounded triangle (Figs. 3 and 4) to an elongated structure with an S-shaped anterior margin (Figs. 5, 8, 9). Occasionally provided with a flat appendix situated between oral disc and upper body wall (Figs. 6, 7, 10–12). Micronucleus ellipsoidal, near left anterior angle of macronucleus.

Dimensions of *D. rangiferi*, based on measurements of 25 specimens from the rumen contents of reindeer No. 1, are summarized in Table I. Dogiel gives

TABLE I

DIMENSIONS OF *D. rangiferi* FROM THE RUMEN OF REINDEER NO. 1

	$M \pm \sigma_M$	σ	C.V., %	Observed limits of variation
Length	201.40 ± 4.45 μ	±22.25 μ	±11.05	156–258 μ
Width	165.96 ± 2.70 μ	±13.50 μ	±8.13	144–204 μ
Length/width ratio	1.2 ±0.01	±0.05	±4.10	1.08–1.37
Length of longit. part of macronucleus	110.88 ± 3.38 μ	±16.92 μ	±15.24	72–147 μ
Length of transverse part of macronucleus	58.08 ± 1.60 μ	±8.01 μ	±13.82	45–72 μ
Length of caudal lobe	9.48 ± 0.86 μ	±4.29 μ	±45.00	3–21 μ

the following dimensions of *Diplodinium rangiferi* (his *D. r. major*): length 166 (128–210) μ, width 136 (110–165) μ, length to width ratio 1.22. Thus *D. rangiferi* from the faunule of the reindeer No. 1 examined by us were larger than those studied by Dogiel.

Variation

The body shape of *D. rangiferi* is comparatively constant, the length of the right caudal lobe varying but slightly. The shape of the macronucleus is, however, very variable (Figs. 3–12). There exists a direct correlation between the size of the body, size of the macronucleus, and the complexity of its shape, large nuclei being of a more complicated shape than the small ones.

Occurrence

D. rangiferi was found in 12 out of 14 faunules examined and constituted 3.2 (2–5)% of the ophryoscolecid ciliates.

Relationships

Dogiel (3) pointed out the close resemblance of this species to *Diplodinium costatum major* from the African antelope *Rhaphiceros* sp. Discussing the relationship of *D. rangiferi major* to *D. costatum major*, he wrote (translated from German):

> "...if these two species would not occur in so remotely related and geographically isolated animals, I would have been inclined to assign them to the same species" (3, p. 62). However, in his monograph of the family Ophryoscolecidae, published two years later (4) he regarded them as separate species.

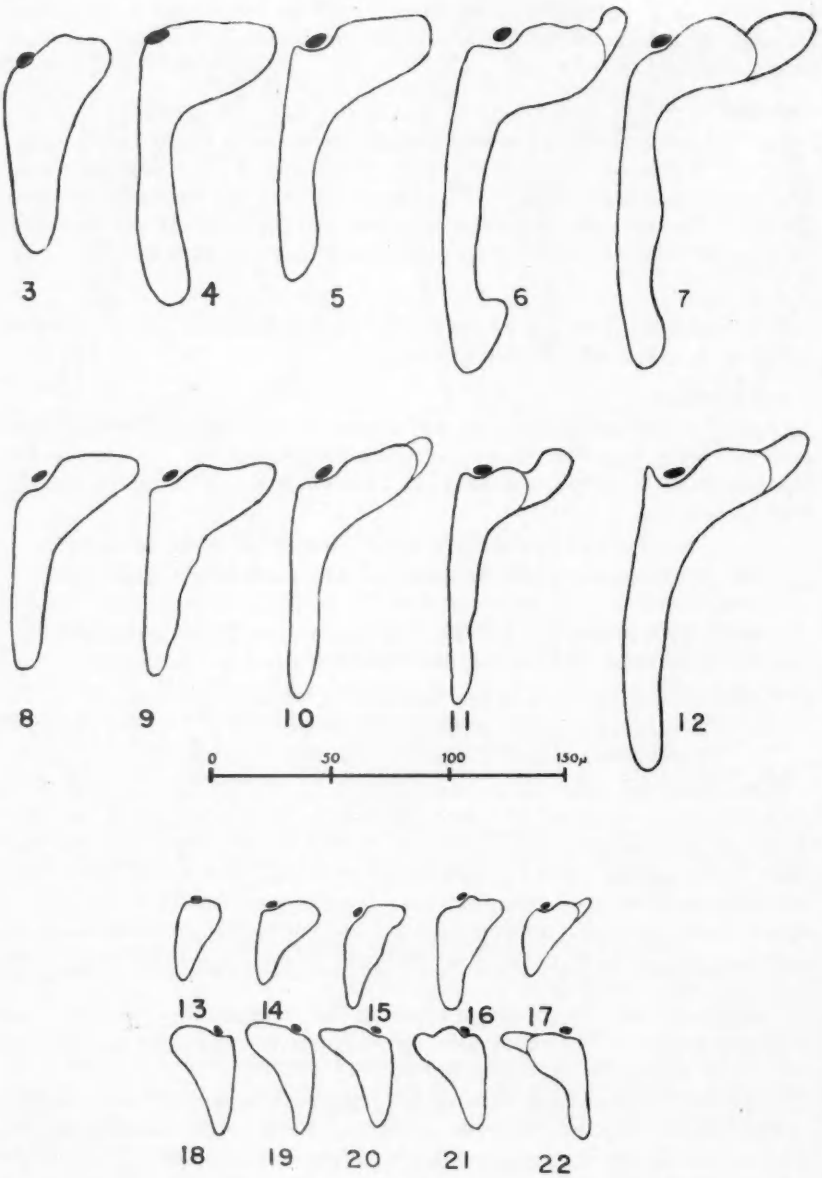
2. *Diplodinium dogieli* Kofoid and MacLennan, 1930 (Figs. 13–24)
Syn.: *Diplodinium rangiferi minor* (3: 51–52, Fig. 10)
Anoplodinium rangiferi minor (4:101, Fig. 54), (6:147)

Body ellipsoidal with truncated anterior end, 97 (84–108) μ long, 72 (60–81) μ wide; length to width ratio 1.35 (1.22–1.47). Operculum small, near the middle of anterior body side. Oral area slanted to left. Right and left body sides nearly equally convex; posterior end rounded; right caudal lobe inconspicuous, rounded, not exceeding 6 μ. Longitudinal cuticular fold on left side of body between nuclear apparatus and contractile vacuoles; near the posterior pole of body it bends to the right to form the upper wall of the groove in which anus opens.

Endoplasmic sac large, not penetrating into operculum. Rectum at an angle of about 70–80° to body axis, opening near posterior pole of body into the caudal end of the left lateral furrow.

Two contractile vacuoles between left lateral furrow and left body margin.

Macronucleus triangular, with rounded ends, its right margin concave, left margin slightly convex, its anterior margin with depression in which the micronucleus is situated. In some specimens the anterior end of the macronucleus has a small, flattened appendix directed to the right and situated between the oral area and body wall (see Figs. 17 and 22).



FIGS. 3–22. Outlines of the nuclear apparatus of 10 specimens of: Figs. 3–12, *D. rangiferi:* Figs. 13–22, *D. dogieli.*

TABLE II

DIMENSIONS OF *D. dogieli* FROM THE RUMEN OF REINDEER NO. 1

	$M \pm \sigma_M$	σ	C.V., %	Observed limits of variation
Length	97.08±1.58 μ	±7.96 μ	±8.21	84–108 μ
Width	72.16±1.01 μ	±5.07 μ	±7.04	60–81 μ
Length/width ratio	1.35±0.02	±0.08	±5.64	1.22–1.47
Length of macronucleus	41.84±1.0 μ	±5.22 μ	−12.72	33–51 μ
Width of macronucleus	29.68±0.61 μ	±3.03 μ	±10.10	24–36 μ
Caudal lobe	3.00±0.20 μ	±1.09 μ	−36.00	0–6 μ

Micronucleus ellipsoidal, in depression of anterior margin of macronucleus near lateral cuticular fold.

Dimensions of *D. dogieli*, based on measurements of 25 stained and mounted specimens from the rumen of reindeer No. 1, are summarized in Table II. Dogiel (4) gives the following dimensions for this form: length 89 (70–110) μ, width 69 (48–77) μ, length to width ratio 1.29. The population of *D. dogieli* under consideration was characterized thus by slightly larger body size than that examined by Dogiel.

Variation

Individuals of this species are fairly constant in their body shape. The shape of their macronuclei varies considerably (Figs. 13–22). Many specimens have macronuclei provided with appendages situated between the oral disc and the body wall (Figs. 17, 22, 24).

Occurrence

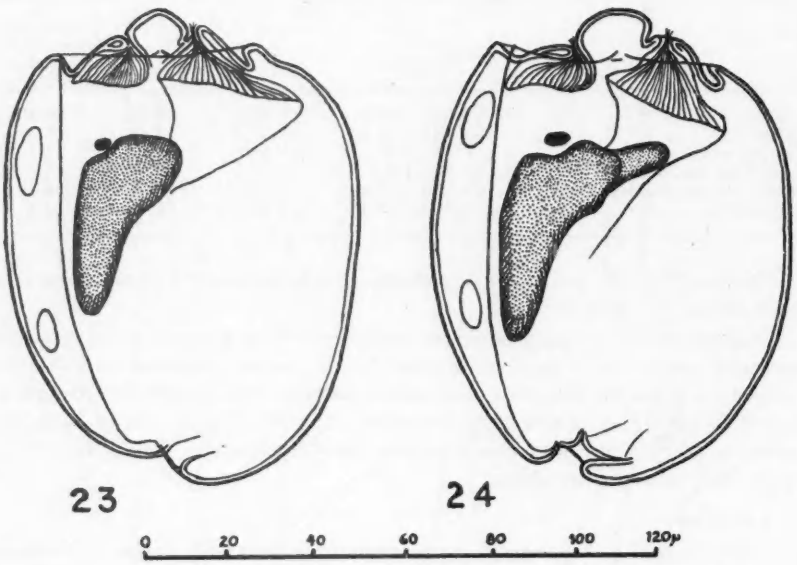
Diplodinium dogieli was present in 13 out of 14 reindeer examined and constituted 2.5(0.1–6.0)% of the ophryoscolecid ciliates.

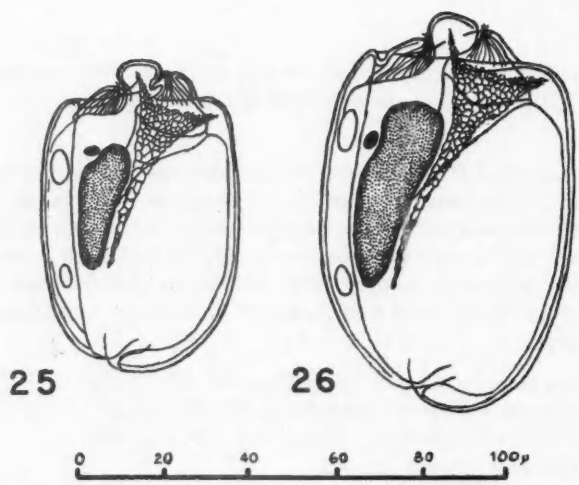
Relationships

Dogiel (3,4) regarded *D. dogieli* (his *Diplodinium rangiferi* forma *minor*) as conspecific with *Diplodinium rangiferi* (his *D. rangiferi* forma *major*). However, he himself has pointed out the absence of intermediate forms between his two "forms" of *D. rangiferi*, nor was I able to find such forms in my material. This compels me to agree with Kofoid and MacLennan (8), who regard *D. rangiferi* Dogiel, 1925 and *D. dogieli* Kofoid and MacLennan, 1932 as separate species.

3. *Eremoplastron impalae* (Dogiel, 1925) (Figs. 25–27), (8:99)
Syn.: *Diplodinium neglectum* f. *impalae* (2:52–53, Fig. 11)
Eudiplodinium neglectum f. *impalae* (4:110, Fig. 60)
Eremoplastron tarandi (8:100)

Body ellipsoidal, truncated anteriorly, 78(66–96) μ long, 56(48–69) μ wide; length to width ratio 1.43 (1.33–1.53). Right and left body sides equally convex. In contracted specimens operculum situated near middle of anterior





FIGS. 23 and 24. *D. dogieli* Kofoid and MacLennan, 1932, from the upper side.
FIGS. 25 and 26. *Eremoplastron impalae* (Dogiel, 1925), from the upper side.

body end. Oral disc slanting to the right. Posterior end rounded, with inconspicuous caudal lobe 0–6 μ long. A narrow, hardly discernible cuticular fold on the upper body surface between nuclear apparatus and contractile vacuoles. Near posterior pole of body it forms a shallow depression in which anus opens.

Endoplasmic sac not penetrating into operculum. Oesophagus parallel to skeletal plate. Rectum comparatively narrow, at an angle of about 70–80° to main body axis.

Two contractile vacuoles between nuclear apparatus and left body side, approximately on the levels of anterior and posterior ends of macronucleus.

Macronucleus club-shaped to cuneiform. Micronucleus in a depression of anterior fourth of macronucleus, between it and cuticular fold.

One cuneiform skeletal plate with wide triangular base and narrow body, terminating in an acute apex. Its left anterior corner in base of operculum, its right anterior corner near right anterior body angle. Cuneiform body of skeletal plate close to the right margin of macronucleus, terminating near its caudal end.

Measurements of 25 stained and mounted specimens from the faunule of reindeer No. 1 are summarized in Table III. According to Dogiel (3, 4) the dimensions of *E. impalae* from the reindeer are: length 85 (74–105) μ, width 62(60–72) μ, length to width ratio 1.36; specimens from the antelope *Aepyceros* sp.: length 76(60–90) μ, width 47(39–60) μ, length to width ratio 1.6.

TABLE III

DIMENSIONS OF *Eremoplastron impalae* FROM REINDEER No. 1

	$M \pm \sigma_M$	σ	C.V., %	Observed limits of variation
Length	78.36±1.48 μ	±7.41 μ	±9.50	66–96 μ
Width	56.40±1.03 μ	±5.16 μ	±9.15	48–69 μ
Length/width ratio	1.43±0.03	±0.13	±8.22	1.33–1.53
Length of macronucleus	39.48±1.19 μ	±5.97 μ	±12.63	30–51 μ
Width of macronucleus	15.00±0.24 μ	±1.20 μ	±8.00	12–18 μ
Length of caudal lobe	4.20±0.31 μ	±1.53 μ	±36.43	0–6 μ

Variation

The body shape of *E. impalae* is fairly constant. The position of the micronucleus is one of the most variable characters of this species. Although it is mostly situated on the level of the anterior end of the macronucleus (Fig. 25), its position varies within the limits of the anterior half of this last (Fig. 26). In about 90% of individuals it is found on the level of the anterior third of the macronucleus. Figure 27 represents this variability in the faunule of the reindeer No. 1. The variants are grouped into four groups: the first group comprises individuals with the micronucleus situated on the level of the anterior pole of the macronucleus. In individuals of the second

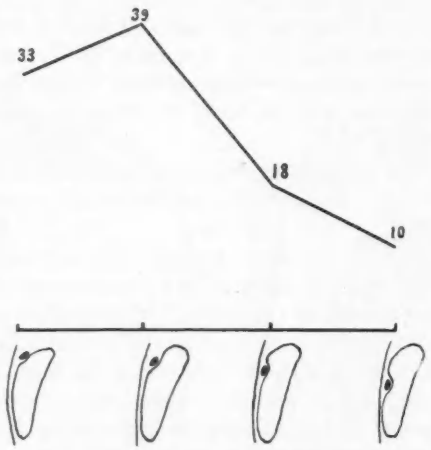


FIG. 27. Variation of the nuclear apparatus of *E. impalae* from the reindeer No. 1.

group the micronucleus is situated below the level of the anterior pole, but the depression of the surface of the macronucleus, in which the micronucleus is situated, is open anteriorly. In individuals of the third group the micronucleus is shifted farther caudal and the groove, in which it is situated, is delimited anteriorly by a prominence of the macronucleus. The fourth group contains individuals whose micronuclei are situated near the middle of the macronucleus. Such individuals were seen already by Dogiel (3), who states that they are rare, 95% of *E. impalae* possessing micronuclei situated near the anterior part of the macronucleus. In the population of the reindeer No. 1, 72% of individuals of *E. impalae* belonged to the first two groups, in which the micronuclei are situated on the level of the anterior end of the macronucleus; only 10% of individuals had micronuclei situated near its middle.

Occurrence

E. impalae was present in 12 out of 14 faunules examined and constituted 7.4(3–21)% of the ophryoscolecid ciliates. It was thus the most abundant species of the higher Ophryoscolecidae of reindeer.

Relationships

Dogiel (2) described *E. impalae* (his *Diplodinium neglectum* f. *impalae*) from the Impala antelope (*Aepyceros melampus*), and later found this species in European reindeer. According to Dogiel, the dimensions of specimens from the antelope are 76(60–90) μ × 47(39–60) μ with a length to width ratio of 1.6, those of specimens from the reindeer 85(74–105) μ × 62(60–72) μ, length to width ratio 1.36. Kofoid and MacLennan (8) regarded specimens from the reindeer as a distinct species and have named it *Eremoplastron tarandi*. They stated that "The specimens from *Rangifer tarandus* are relatively shorter; a cuticular line is present near the dorsal edge of the macronucleus, and the anterior end of the macronucleus is relatively broad. The

important morphological differences, and the wide difference in hosts and distribution make it impossible to include this ciliate from *Rangifer* with *E. impalae*, and accordingly we place it in a separate species, *E. tarandi*" (8, p. 99). The present specimens are intermediate in size and proportions between Dogiel's specimens from reindeer and those from the antelope (see above). The width of the anterior end of the macronucleus in our material varied considerably (see Figs. 25–27), and therefore cannot be regarded as a reliable taxonomic character. The absence of the "cuticular line" (fold) near the "dorsal" edge of the macronucleus of *E. impalae* from the antelope in Dogiel's Figs. 4D (2, p. 128) and 60*b* (4, p. 110) cannot be construed as indicating the absence of the "line" in his specimens, because Dogiel has not shown it in his drawings of many species in which it does exist, as for example in *E. spectabile* (cf. Figs. 28 and 29 herein with Dogiel's figures of *E. neglectum spectabile* (3, p. 53, Figs. 12 and 4, p. 109, Fig. 59). Furthermore, this line is present in one of Dogiel's drawings of *A. denticulatum quinquespinosum* (4, Fig. 46*a*, p. 87), and absent in the other (4, Fig. 46*b*), despite the fact that it is very conspicuous in this species. Available data are thus inadequate for the differentiation of *Eremoplastron tarandi* Kofoid and MacLennan, 1932 from *E. impalae* (Dogiel, 1925). This compels me to regard *Eremoplastron tarandi* Kofoid and MacLennan, 1932 as a synonym of *E. impalae*.

Dogiel in 1925 (3) described *E. impalae* and *E. spectabile* as forms of one species: *Diplodinium neglectum impalae* and *D. neglectum spectabile*. The relation between these forms, which Kofoid and MacLennan regard as separate species, is discussed hereunder.

4. *Eremoplastron spectabile* (Dogiel, 1925) (Figs. 28 and 29)

Syn.: *Diplodinium neglectum spectabile* (3:53, Fig. 12)
Eudiplodinium neglectum spectabile (4:109, Fig. 59)
Eremoplastron spectabile (8:99)
E. neglectum spectabile (6:143,147)

Body ellipsoidal, anterior end truncated, 105 (93–117) μ long, 78(69–90) μ wide; length to width ratio 1.35(1.25–1.45). Operculum comparatively small, in contracted specimens near middle of anterior body margin, in expanded specimens shifted to the left. Oral disc slanted to the right. Right and left body sides in contracted specimens nearly equally convex, in expanded specimens right side nearly flat. Posterior end of body rounded, right caudal lobe inconspicuous, not exceeding 10 μ. Longitudinal fold on upper body side between nuclear apparatus and contractile vacuoles, terminating at caudal end as funnel-shaped depression, in which anus opens.

Endoplasmic sac not penetrating into operculum. Rectum at an angle of about 70–80° to main body axis. Anus slightly to left from posterior pole of body.

Two contractile vacuoles between longitudinal fold and left body edge at levels of anterior and posterior ends of macronucleus.

Macronucleus triangular, with rounded ends, about half as long as body. Micronucleus ellipsoidal, on the level of its middle third.

TABLE IV

DIMENSIONS OF *Eremoplastron spectabile* FROM REINDEER No. 1

	$M \pm \sigma_M$	σ	C.V., %	Observed limits of variation
Length	104.96±1.42 μ	±7.11 μ	±6.77	93–117 μ
Width	78.36±1.54 μ	±7.71 μ	±9.86	69–90 μ
Length/width ratio	1.35±0.01	±0.06	±4.44	1.25–1.45
Length of macronucleus	55.30±1.70 μ	±8.52 μ	±15.40	42–69 μ
Width of macronucleus	21.24±1.74 μ	±8.88 μ	±4.21	18–27 μ
Length of caudal lobe	6.60–0.45 μ	−2.34 μ	035.45	0–10 μ

Skeleton cuneiform, two-thirds of body length. Its left anterior corner in the operculum, right anterior corner near right body side, at base of outer adoral lip. Its posterior end behind posterior pole of macronucleus.

Measurements of 25 specimens of *E. spectabile* from the rumen of reindeer No. 1 are summarized in Table IV. Dogiel gives the following measurements of *E. spectabile*: length 133 (115–150) μ, width 91 (83–101) μ, length to width ratio 1.46. Thus his type specimens of *E. spectabile* were larger than specimens examined by us. Besides that, the type specimens were more elongate, their length to width ratio being 1.46. However, the measurement of Dogiel's Fig. 12 (3, p. 53) shows that the length to width ratio of this specimen is 1.30, whereas that of the specimen represented on his Fig. 59 (4, p. 109) is 1.28, thus smaller than the average length to width ratio of our specimens, although within the limits of variation observed in our material.

Variation

The body shape of *E. spectabile* is comparatively constant. The level on which the micronucleus is situated varies within the limits of the middle third of the macronucleus. Although usually situated near its middle, it can often be found on the level of the anterior or posterior end of the middle third of the macronucleus (see Figs. 28 and 29).

Occurrence

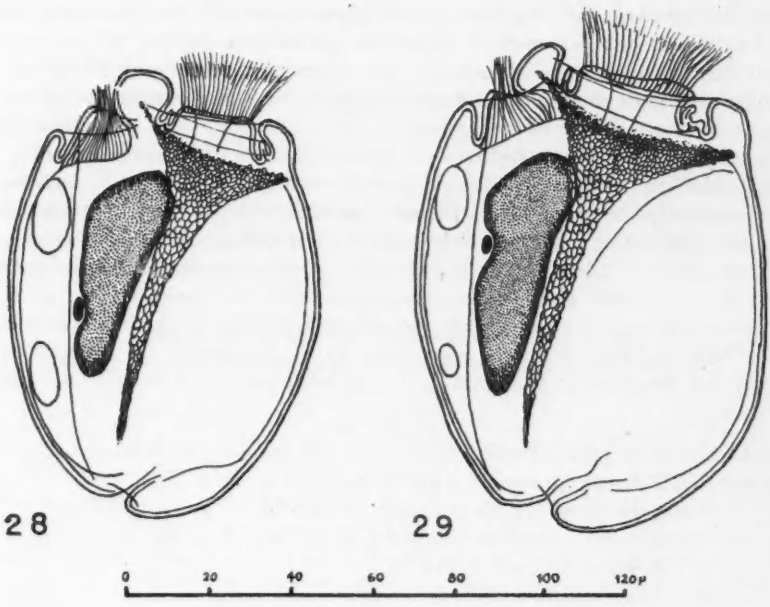
E. spectabile was present in 12 out of 14 faunules examined and constituted 2.8 (0.1–6.0)% of the ophryoscolecid ciliates.

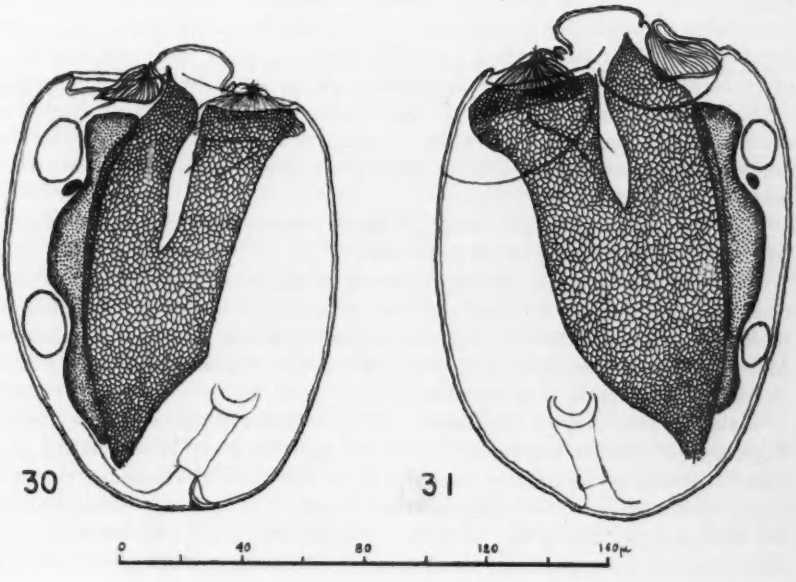
Relationships

E. spectabile was described by Dogiel (3) as a form of his *Diplodinium neglectum*: *D. neglectum spectabile*. Discussing it (3, p. 53), he writes: "In most of its characters it is identical with the first form (*D. neglectum impalae*, Auct.) but differs from it in its larger size and another position of the micronucleus".

FIGS. 28 and 29. *Eremoplastron spectabile* (Dogiel, 1925) from the upper side. Note the variation in the position of the micronucleus.

FIGS. 30 and 31. *Metadinium magnum* (Dogiel, 1925): Fig. 30, from the upper side; Fig. 31, from the lower ("left") side.


28
29
0 20 40 60 80 100 120μ


30
31
0 40 80 120 160μ

Later, in 1927 (4, p. 109), he added to this characteristic the peculiarity of the shape of the macronucleus of *D. neglectum spectabile* in which "the anterior end of the macronucleus is dilate more than in other hitherto described forms". Another character in which *E. spectabile* differs from *E. impalae*, as well as from many other species of *Eremoplastron*, is the size and shape of its skeletal plate. The posterior end of this plate usually extends in *E. spectabile* somewhat behind the posterior end of the macronucleus, whereas in *E. impalae* it terminates in its vicinity. Moreover, the skeletal plate of *E. spectabile* is wider than that of *E. impalae;* on the level of the anterior pole of the macronucleus its width in the first species exceeds 7 μ, whereas in *E. impalae* it is about 4–5 μ. When viewed from the upper side, the skeletal plate of *E. spectabile* is often superimposed on the anterior portion of the right margin of the macronucleus, which is never the case in *E. impalae*. I thus follow Kofoid and MacLennan in regarding *E. spectabile* and *E. impalae* as distinct species.

5. *Metadinium magnum* (Dogiel, 1925) (Figs. 30 and 31), (8:116)
   Syn.: *Diplodinium ypsilon magnum* (3:55–56, Fig. 14, p. 55)
   Eudiplodinium ypsilon magnum (4:129–130, Fig. 72, p. 129)
   Ostractodinium ypsilon magnum [*sic*] (6:143–144, 145)
   O. ypsilon major [*sic*] (6:144)
   E. ypsilon magnum (6:147)

Body short, wide, nearly quadrangular, without caudal lobes, 174 (138–231) μ long, 132 (105–162) μ wide; length to width ratio 1.33 (1.2–1.5). Operculum comparatively small, flat, near middle of anterior body margin. Right and left body sides equally convex. Posterior end smoothly rounded, often with a nipple-like projection near its middle, where anus is situated. Left lateral fold rudimentary, extending from anterior margin of outer lip of metoral membranelle zone approximately to the level of micronucleus.

Oral and metoral areas nearly at right angles to main body axis. Endoplasmic sac closely applied to body walls. Rectum nearly semicircular, its proximal portion almost at right angles to main body axis, its distal portion nearly coinciding with this axis.

Two contractile vacuoles, anterior much larger than posterior, situated between macronucleus and left body edge.

Macronucleus long and narrow, its length to width ratio 7 (5–9). Its anterior end slightly dilate, its anterior surface flattened. Left side of macronucleus with two wide shallow depressions accommodating contractile vacuoles. Micronucleus ellipsoidal, caudally from anterior contractile vacuole, in anterior depression of macronucleus.

Skeletal plate single, Y-shaped (hence Dogiel's name for this species: *Diplodinium ypsilon magnum*), about half as wide as body, extending from base of operculum nearly to caudal end of body. Right anterior corner of plate produced into a round prominence reaching right body margin near the base of outer adoral lip. Anterior two thirds of plate with nearly parallel right and left side; posterior third abruptly tapering caudally, with rounded

posterior end. Median longitudinal fissure extends from the anterior edge of plate to its middle, subdividing it into a narrower left and a wider right portion.

Food consists predominantly of long plant fibers, which often badly distort the body.

Measurement of 25 stained and mounted specimens from the faunule of the reindeer No. 2 were as follows (see Table V). According to Dogiel (3, 4) *M. magnum* is 183 (156–201) μ long and 111 (92–135) μ wide, with a length to width ratio of "about 1.64". His specimens were thus slightly more elongated than ours.

TABLE V

DIMENSIONS OF *Metadinium magnum* FROM REINDEER No. 2

	$M \pm \sigma_M$	σ	C.V., %	Observed limits of variation
Length	174.4 ±3.40 μ	±18.99 μ	±10.92	138–231 μ
Width	131.6 ±2.21 μ	±11.07 μ	±8.41	105–162 μ
Length/width ratio	1.33±0.01	±0.07	±5.37	1.2–1.5
Length of macronucleus	115.9 ±2.56 μ	±12.78 μ	±11.04	96–135 μ
Width of macronucleus	15.0 ±0.43 μ	±2.16 μ	±14.00	12–18 μ

Variation

This species is fairly constant in its shape and proportions, but varies considerably in size. The smallest specimen seen in the faunule of reindeer No. 2 was 138 × 105 μ, the largest 231 × 162 μ.

Occurrence

M. magnum was present in 12 out of 14 faunules examined and never exceeded 2.5% of the ophryoscolecid ciliates.

Relationships

This species is closely related to other species of the genus *Metadinium*, including those with two skeletal plates, as for example *M. medium*. Kofoid and MacLennan (8) describe its skeleton as two skeletal plates fused posteriorly. The macronucleus of *M. medium*, the sole species of this genus possessing two skeletal plates, is displaced to the left and pressed in between the vacuoles deeper than in species with "fused" plates, as *M. tauricum* (9, Fig. 8) and *M. magnum*. *M. medium* is also larger than other species of this genus, and has to be regarded as evolutionary more advanced species. Therefore I regard the skeletal plate of *M. magnum* as one plate in the process of fission, *not* as two plates in the process of fusion. According to Kofoid and MacLennan (8, p. 133) *Ostracodinium crassum* Dogiel "is separated from the other species of *Ostracodinium* by its relatively short and broad body and by the narrowness of its skeletal plate". This species is thus referable to *Metadinium*, and, having a single entire skeletal plate, is a primitive, if not the most primitive, species of this genus.

6. *Enoploplastron triloricatum* (Dogiel, 1925) (see ref. 11, Fig. 3), (8:141–142, Fig. 41, p. 110)

Syn.: *Diplodinium triloricatum* (2:133–134, Fig. 6B)
Diplodinium triloricatum triloricatum (3:56, Pl. II, Fig. 16)
Ostracodinium triloricatum f. *triloricatum* Dog. (1925) (4:152–154, Figs. 87*a*, *b*), (6:143, 147).

This species was described by Dogiel from the steenbock antelope and later found by him in reindeer, sheep, and cattle. It is one of the most common species of the Ophryoscolecidae—its full synonymy would involve a better part of the literature on the systematics of this family. The above references are of necessity limited to papers proposing important nomenclatorial changes or reporting the finding of this species in reindeer. Dimensions of 25 specimens of this species from the rumen of the reindeer No. 2 were as follows (see Table VI). According to Dogiel (4) dimensions of *E. triloricatum* from the reindeer

TABLE VI

DIMENSIONS OF *Enoploplastron triloricatum* FROM REINDEER NO. 2

	$M \pm \sigma_M$	σ	C.V., %	Observed limits of variation
Length	$86.64 \pm 1.14\,\mu$	$\pm 5.58\,\mu$	± 6.41	$78–96\,\mu$
Width	$50.28 \pm 0.64\,\mu$	$\pm 3.21\,\mu$	± 6.01	$45–57\,\mu$
Length/width ratio	1.75 ± 0.03	± 0.15	± 8.57	1.60–1.88

are: length 90 (75–103) μ, width 47 (40–58) μ, length to width ratio 1.90; specimens from the antelope (*Rhaphiceros*) being 82 (60–110) μ long and 45 (37–56) μ wide, with the length to width ratio of 1.8. Specimens from the cattle were, according to Dogiel, 100 (85–112) μ long, 61 (51–70) μ wide, their length to width ratio 1.64.

Occurrence

In our material this species was present in 12 out of 14 faunules examined and constituted 6.6 (2.5–11)% of the ophryoscolecid ciliates.

Relationships

In a previous paper (10) I have pointed out the similarity of this species to *Epidinium* and regarded *Enoploplastron* as being closely related to the ancestors of *Epidinium* and thus to those of the entire subfamily Ophryoscolecinae.

7. *Enoploplastron confluens* (Dogiel, 1925) (Figs. 32 and 33), (8:142)

Syn.: *Diplodinium triloricatum confluens* (3:56–57, Pl. II, Fig. 17*a*, *b*)
Ostractodinium triloricatum forma *confluens* (4:154–155, Fig. 88*a*, 88*b*), (6:143–147)

Body a wide, flattened ellipsoid, 133(111–156) μ long, 105(93–114) μ wide; length to width ratio 1.27 (1.09–1.41). Operculum long and flat, metoral membranelle zone shifted slightly caudad, its base in contracted specimens

caudad to anterior pole of macronucleus. Left body side more convex than right side. Posterior end rounded, caudal lobes absent. Left longitudinal cuticular fold between nuclear apparatus and contractile vacuoles.

Endoplasmic sac not penetrating into operculum. Anus near posterior pole of body.

Two contractile vacuoles between nuclear apparatus and left body edge on the levels of anterior and posterior ends of macronucleus. In recently divided specimens caudal vacuole sometimes absent (Fig. 33).

Macronucleus spindle-shaped, with rounded ends and a depression on its left side, accommodating micronucleus.

Skeletal plate very wide and long, extending from base of operculum nearly to caudal end and from macronucleus to right body side, where it transgresses to the lower side of the body, curving to the left just below operculum and almost reaching left margin of plate. Anterior third to half of the plate with a median pear-shaped aperture, beginning at its anterior margin and widening caudally. (*In E. triloricatum*, aperture spindle-shaped.) Similar aperture on the right side of skeletal plate, hardly discernible when viewed from upper side.

TABLE VII

DIMENSIONS OF *Enoploplastron confluens* FROM REINDEER No. 2

	$M \pm \sigma_M$	σ	C.V., %	Observed limits of variation
Length	133.3 ±1.76 μ	±9.66 μ	±7.26	111–156 μ
Width	105.2 ±1.68 μ	±9.21 μ	±8.77	93–114 μ
Length/width ratio	1.27±0.01	±0.07	±5.51	1.09–1.41
Length of macronucleus	74.3 ±1.73 μ	±9.01 μ	±12.13	48–93 μ

Food: Besides spores, yeast, and small pieces of cellulose, *E. confluens* ingests long pieces of mycelium, which it winds up spirally.

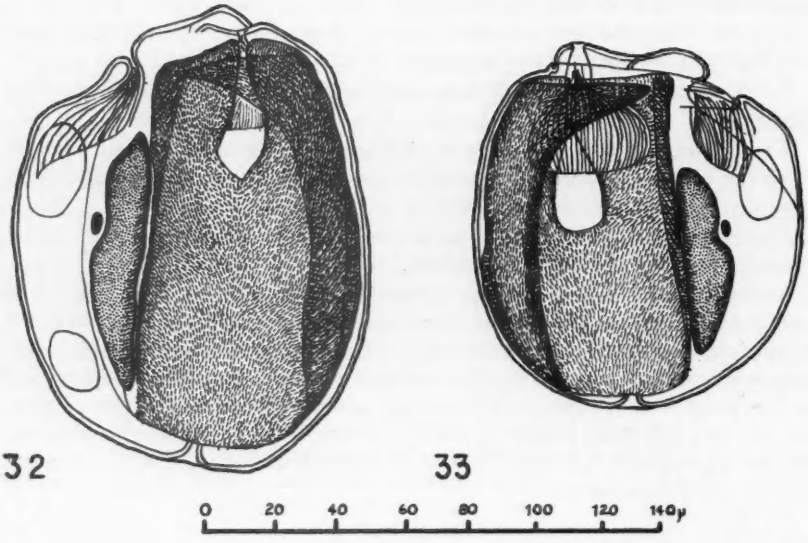
The measurements of 30 specimens from the rumen of the reindeer No. 2 were as follows (see Table VII). According to Dogiel (3, 4) the dimensions of *E. confluens* (his *Ostracodinium triloricatum confluens*) are: length 138 (120–157) μ, width 86 (72–103) μ, length to width ratio 1.6. His type specimens were thus more elongate than those examined by us.

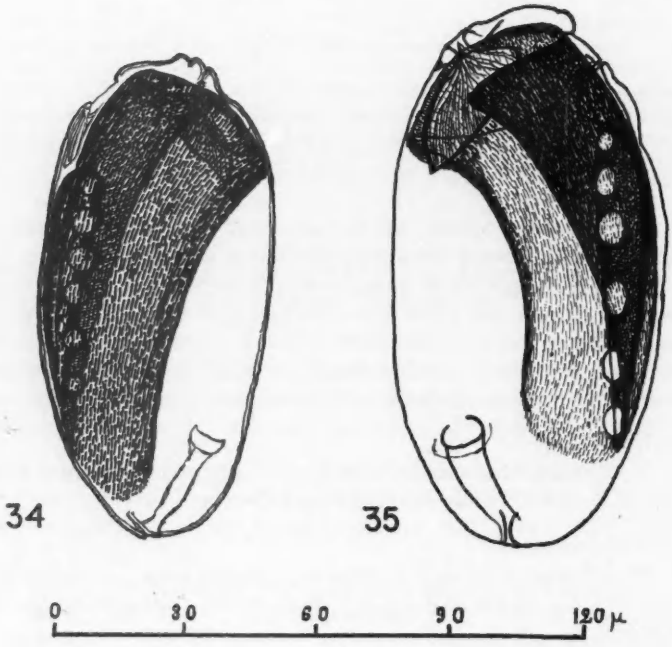
Occurrence

E. confluens was present in 8 out of 14 reindeer examined and constituted 3.1(0.5–4.5)% of the ophryoscolecid ciliates.

Relationships

E. confluens is closely related to *E. triloricatum*. Intermediate forms not occurring, I follow Kofoid and MacLennan in regarding them as separate species.


32
33
0 20 40 60 80 100 120 140μ


34
35
0 30 60 90 120 μ

8. *Ostracodinium obtusum* Dogiel and Fedorowa, 1925 (Figs. 34 and 35), (8:133).

Syn.: *Diplodinium dentatum* Fior. var. *obtusum* (7:101, Fig.5)
Diplodinium obtusum obtusum (4:136–137, Fig. 76), (6:147)

Body ellipsoidal, 108 (93–120) μ long, 56 (45–63) μ wide; length to width ratio 1.93 (1.70–2.05). Right and left body sides but slightly convex; posterior end rounded. Caudal lobes absent. Oral area slanted to the right and to lower side, metoral membranelle zone anterior to anterior pole of macronucleus, nearly in contact with it. Operculum long, flat, extending between oral and metoral zones.

Anus slightly to the right from the posterior pole of the body.

Four contractile vacuoles in young individuals, six in individuals preparing for division. Macronucleus pressed tightly to left body wall, displacing the vacuoles to lower side of the body. At least the middle four vacuoles in depressions of macronucleus.

Macronucleus club- to sausage-shaped, with a wider anterior end, about six to seven times as long as wide. Its anterior pole at a considerable distance from anterior end of body. Micronucleus below macronucleus near the middle of body.

Skeletal plate one, semicylindrical in its posterior portion, underlying three fourths of body wall in its anterior portion. Its right edge runs from right side of base of outer adoral lip to posterior pole of body. Its left edge runs from middle of anterior end of lower body side to posterior pole of macronucleus. About two thirds of skeletal plate is situated under upper surface of body, one third under its lower surface. Boundary between these portions in contact with macronucleus. Anterior margin of the plate runs from the right side of the base of the outer adoral lip anticlockwise under the upper body wall below the base of the operculum and under metoral membranelle zone and continues to the lower body side, where it ends slightly to the right from the mid-line, encircling about three quarters of the body.

This species often engulfs long pieces of cellulose and rolls them up in the endoplasm, which often results in enormous distortion of the body.

Measurements of 25 stained and mounted specimens from the rumen of the reindeer No. 10 are summarized in Table VIII. According to Dogiel (3, 4) specimens of this species from the reindeer measure 134 (118–148) $\mu \times$ 67 (55–80) μ, with a length to width ratio of 2, whereas specimens from cattle are smaller: 122 (107–133) $\mu \times$ 58 (54–61) μ. Our specimens are even smaller than the specimens from cattle examined by Dogiel.

FIGS. 32 and 33. *Enoploplastron confluens* (Dogiel, 1925): Fig. 32, from the upper side; Fig. 33, from the lower side.
FIGS. 34 and 35. *Ostracodinium obtusum* (Dogiel and Fedorowa, 1925): Fig. 34, from the lower side; Fig. 35, from the upper side.

TABLE VIII

DIMENSIONS OF *Ostracodinium obtusum* FROM REINDEER No. 10

	$M \pm \sigma_M$	σ	C.V., %	Observed limits of variation
Length	107.64±1.52 μ	±7.62 μ	±7.04	93–120 μ
Width	55.80±0.76 μ	±3.82 μ	±6.82	45–63 μ
Length/width ratio	1.93±0.02	±0.08	±4.15	1.70–2.05
Length of macronucleus	57.48±1.29 μ	±6.45 μ	±11.32	45–72 μ
Width of macronucleus	11.72±0.58 μ	±2.88 μ	±24.62	6–18 μ

Variation

This species seems to be fairly constant in its morphological characters. Peculiar is the complete absence of forms with well-developed caudal lobes, which Dogiel has found in cattle (*O. obtusum monolobum* and *O. obtusum dilobum*) and which he regarded as conspecific with *O. obtusum* (his *O. obtusum obtusum*).

Occurrence

This species was present in 12 out of 14 faunules examined and constituted 1 or less % of the ophryoscolecid ciliates, with the exception of one faunule, where it constituted 3%.

Relationships

I follow Kofoid and MacLennan, who regard *O. obtusum* as a separate species, related to *O. monolobum* and *O. dilobum* occurring in cattle.

9. *Polyplastron arcticum* sp. n. (Figs. 36–39)

Syn.: "*Polyplastron multivesiculatum* from reindeer" (6:144, 147)

Diagnosis

Polyplastron: body a wide ellipse, 1.3 (1.14–1.45) times as long as wide. Caudal lobe inconspicuous. Macronucleus curved, nearly reaching the posterior end of body. No vacuoles between primitiva and carina, one or two vacuoles near right body margin. Two plates on lower side of body; one narrow plate near its right margin, one long plate beginning near base of operculum and reaching left body edge in its middle third. Anticarina absent.

Type host

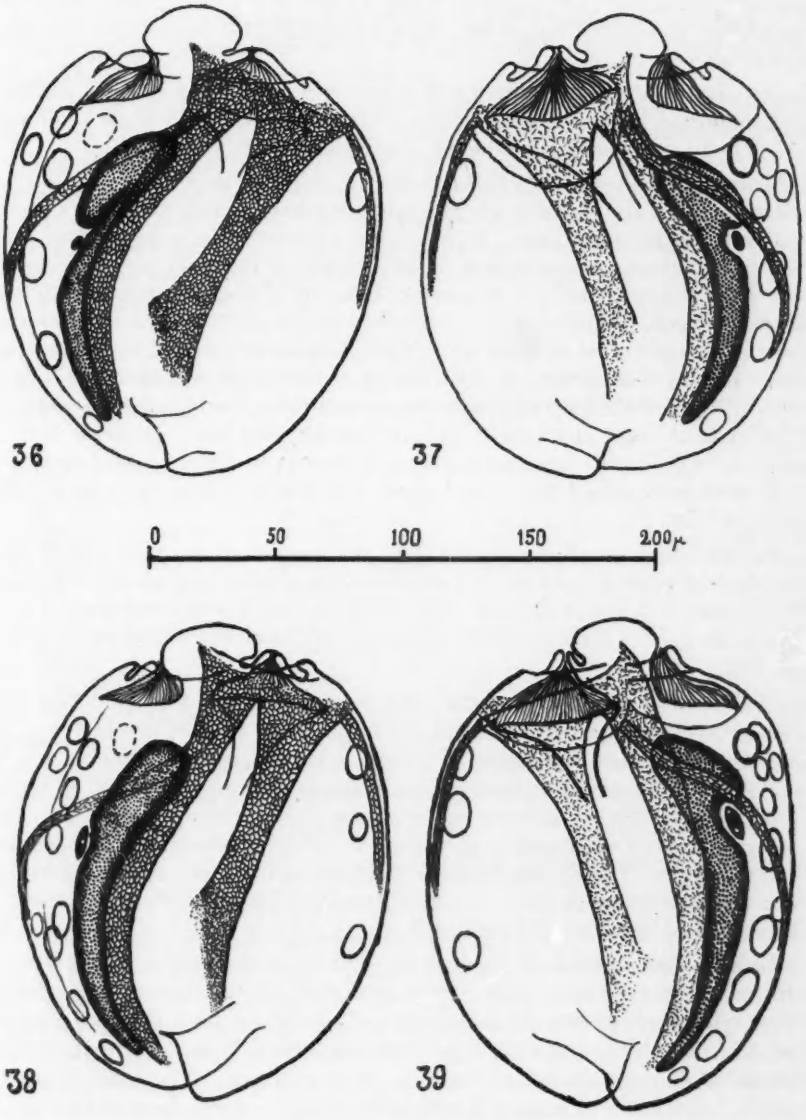
Rangifer tarandus L.

Type locality

Aklavik, N.W.T., Canada.

Description

Body a wide ellipse 183 (147–219) μ long, 141 (123–175) μ wide; length to width ratio 1.3 (1.14–1.45). Oral area slanted to the right, metoral membranelle zone on the level of oral zone. Operculum comparatively large,



FIGS. 36–39. *Polyplastron arcticum* n. sp.: FIGS. 36, 37, young specimens; Figs. 38 and 39, specimens preparing for division.

rounded, at anterior pole of body. Right and left body sides equally strongly convex. Posterior end rounded; right ("ventral") lobe inconspicuous or completely absent, not longer than 9 μ. Longitudinal cuticular fold near left side of macronucleus.

Oesophagus beneath primitiva and carina; it is wide, dilate near the middle, and supported by heavy longitudinal fibrils. Rectum at an angle of about 45° to body axis, opening by the anus near posterior pole of body.

Macronucleus long, club- to hook-shaped, with a dilate anterior portion, curved into an arc of about 70–90°, extending from anterior fourth of body nearly to its posterior pole. Micronucleus ellipsoidal, in a depression near boundary between anterior and middle thirds of macronucleus. In some specimens this depression is deep and oblique, imparting to macronucleus the shape of a hook, similar to that of *Eudiplodinium maggii*. Contractile vacuoles vary in number depending on age. Young daughter animals with a row of four vacuoles near left side of macronucleus. Two more vacuoles close to left body margin on the level of anterior vacuole of this row. Another vacuole on lower side of body just behind metoral membranelle zone, between it and tergum. One or two contractile vacuoles near right side of body, posterior to base of outer adoral lip. Total number of vacuoles in young individuals: eight or nine.

In individuals preparing for division, the number of vacuoles in left row increases to eight and an additional vacuole near anterior end of left margin of posterior daughter is formed. Right vacuole of posterior daughter appears. Thus, the opisthe has one right vacuole; proter, two such vacuoles (see Figs. 38, 39).

Skeleton consists of four plates: two beneath upper surface of body, one near its right side, and one beneath left portion of lower body surface.

Left upper plate ("primitiva" of Dogiel) cuneiform, 12–15 μ wide in its middle portion, curved, extending from base of operculum nearly to posterior pole of body. Its left anterior corner situated in base of operculum, its right anterior corner in the middle of upper side of outer adoral lip, its posterior end near anus. The left side of this plate closely applied to at least the anterior two thirds of macronucleus. Anterior portion of its left side often penetrates between macronucleus and body wall.

Second plate, carina, situated to the right from first plate, parallel to it. Its left anterior corner in close contact with right anterior corner of primitiva. The right anterior corner of the carina is situated near the right body margin in the base of the outer adoral lip. The plate extends posteriorly about two thirds of the body length and terminates in a dilate end with an indistinct posterior margin. This plate in its middle portion is approximately as wide as first plate, thus narrower than both macronucleus and space between middle portions of the two skeletal plates.

Third plate, scutum, very narrow and short, situated near right body margin, extending caudally one third to half body length.

Fourth plate, tergum, beneath left side of lower surface of body, long, 12–15 μ wide in its middle portion, S-shaped. It begins near operculum between bases of outer adoral and metoral lips and runs posteriorly and to the left, crossing macronucleus on the level of its anterior fourth, and reaching left margin of body near its middle.

Bases of all skeletal plates at base of adoral lip, which contains numerous granules of polysaccharides. No rows of skeletal prisms interconnecting bases of lower plates. Anticarina absent. Skeleton of *P. arcticum* thus similar to that of *Elytroplastron;* the new species can be characterized as giant *Polyplastron* with skeleton of *Elytroplastron.*

The measurements of 25 stained and mounted specimens from the reindeer No. 12 were as follows (see Table IX). Dividing specimens measured up to 230 × 180 μ.

TABLE IX

DIMENSIONS OF *Polyplastron arcticum* N. SP. FROM THE REINDEER No. 12

	$M \pm \sigma_M$	σ	C.V., %	Observed limits of variation
Length	183.00±4.12 μ	±20.71 μ	±11.38	147–219 μ
Width	141.12±3.68 μ	±18.38 μ	±13.04	123–175 μ
Length/width ratio	1.29±0.01	±0.05	±3.89	1.14–1.45
Length of macronucleus	115.56±3.24 μ	±16.22 μ	±13.99	87–144 μ
Width of macronucleus	20.04±0.51 μ	±2.53 μ	±12.65	18–21 μ

Variation

The shape of the body of *P. arcticum* varies but slightly. The caudal lobe varies in length, never exceeding, however, 9 μ. The macronucleus varies in shape from club-shaped to hook-shaped, macronuclei of this last type being reminiscent of those of *Eudiplodinium maggii* (see Figs. 38, 39).

Occurrence

Polyplastron arcticum was found only in two reindeer and constituted 3.0 and 1.5% of the ophryoscolecid ciliates.

Relationship and Differentiation from Other Species

Polyplastron arcticum n. sp. has two separate skeletal plates on the upper ("right") side of the body. It shares this character with only two other species of the genus: genotype *P. multivesiculatum* Dogiel and Fedorowa, 1925 and *P. californiensis* Bush and Kofoid, 1948 (1). It differs from both these species in the absence of the anticarina and in the position of the anterior end of the scutum at the right ("ventral") margin of the body. Further characters peculiar to the species under discussion are its larger size and almost complete absence of the caudal lobe. Moreover, *P. arcticum* is relatively much wider than *P. multivesiculatum.* Important taxonomic characters of *P. arcticum* and of related species of this genus are summarized in Table X.

TABLE X

CHARACTERS DIFFERENTIATING *P. arcticum* N. SP. FROM RELATED SPECIES

	P. arcticum n. sp.	Dogiel's "*P. multivesiculatum*" from reindeer(6)	*P. multivesiculatum* Dogiel & Fed. (7)	*P. californiensis* Bush & Kof.(1)
Length	183 μ (147–214) μ	162 μ (up to 240) μ	161 μ (120–190) μ	162 μ (130–200) μ
Width	141 μ (123–175) μ	122 μ (96–174) μ	95 μ (78–140) μ	133 μ (110–155) μ
Length/width ratio	1.29 (1.14–1.45)	1.31	1.7	1.21 (1.11–1.29)
Caudal lobe	Absent	?	Present, small	Present
Anticarina	Absent	?	Present	Present
Vacuoles near the right side of the body	2(proter) 1(opisthe)	?	One	Absent

The absence of anticarina was regarded by Kofoid and MacLennan (8) as diagnostic of their genus *Elytroplastron*. This genus was established for the sole then-known species of Dogiel's subgenus *Diplodinium* (*Polyplastron*)—*D.* (*Polyplastron*) *bubali* Dogiel, 1928 (5), in which the anticarina is absent. This plate is very small and inconspicuous even in *P. multivesiculatum*, where it was first described. The new species is similar to *Elytroplastron bubali* only in the absence of the anticarina and in the considerable size of the tergum, being much closer to *P. multivesiculatum* in other characters. Kofoid and MacLennan undoubtedly overestimated the taxonomic value of the anticarina. It is thus considered that their genus *Elytroplastron* should fall as a synonym of *Polyplastron* (Dogiel) emend. Kofoid and MacLennan, 1932.

The Diplodiniinae of reindeer can be identified with the aid of the following key:

1. Skeleton absent; macronucleus boomerang-shaped or triangular ..... *Diplodinium* Schuberg, 1888 emend. Kofoid and MacLennan, 1932 2
   Skeleton present 3
2(1). Macronucleus boomerang-shaped, body over 140 μ long ..... *D. rangiferi* Dogiel, 1925
   Macronucleus a rounded triangle, body less than 120 μ long ..... *D. dogieli* Kofoid and MacLennan, 1932
3(1). Skeletal plate one, narrow, cuneiform 4
   Several plates or one wide skeletal plate 6
4(3). Macronucleus hook-shaped, micronucleus in the depression of the hook ..... *Eudiplodinium maggii* (Fiorentini, 1889)
   Macronucleus not hook-shaped .... *Eremoplastron* Kofoid and MacLennan, 1932 5
5(4). Micronucleus near the anterior end of the club-shaped macronucleus; body length usually under 95 μ ..... *E. impalae* (Dogiel, 1925)
   Micronucleus near the middle of the triangular macronucleus; body length mostly over 95 μ ..... *E. spectabile* (Dogiel, 1925)
6(3). One wide Y-shaped plate or two skeletal plates. Vacuoles in two wide depressions of the macronucleus ..... *Metadinium* Awerinzew and Mutafowa, 1914
   (In reindeer, one species with a Y-shaped plate .... *M. magnum* (Dogiel, 1925))
   One very wide, not Y-shaped plate, or several plates 7
7(6). One very wide trough-shaped plate without apertures ..... *Ostracodinium* Dogiel, 1925 emend. Kofoid and MacLennan, 1932
   (In reindeer only one species ..... *O. obtusum* (Dogiel and Fedorowa, 1925))
   Several plates, fused or not fused 8
8(7). Three plates fused posteriorly .... *Enoplopiastron* Kofoid and MacLennan, 1932 9
   Four or more plates ..... *Polyplastron* Dogiel, 1925 10

9(8). Apertures between the anterior ends of plates spindle-shaped, length to width ratio of the body > 1.6 ..... *E. triloricatum* (Dogiel, 1925)
Apertures between the anterior ends of plates pear-shaped, length to width ratio of the body < 1.5 ..... *E. confluens* (Dogiel, 1925)
10(8). Anticarina present; length to width ratio about 1.7 ..... *P. multivesiculatum* Dogiel and Fedorowa, 1925
Anticarina absent; length to width ratio 1.3 (1.1–1.5) ..... *P. arcticum* n. sp.

Remarks

To *point 4*: *Eudiplodinium maggii* was found by Dogiel in European reindeer. To *point 8*: The right ("ventral") skeletal plate of *Polyplastron* is very small. To *point 10*: The finding of *P. multivesiculatum* in reindeer by Dogiel (6) was discussed above in connection with *P. arcticum*.

The Ophryoscolecinae of reindeer, as well as the general composition of its fauna of ciliates, will be discussed in a subsequent paper.

References

1. Bush, M. and Kofoid, C. A. Ciliates from the Sierra Nevada bighorn, *Ovis canadensis sierrae* Grinell. U. Calif. (Berkeley) Publ. Zool. **53**, 237–261 (1948).
2. Dogiel, V. Nouveaux infusoires de la famille des Ophryoscolecidés parasites d'antelope africaines. Ann. parasitol. **3**, 116–142 (1925).
3. Dogiel, V. Neue parasitische Infusorien aus dem Magen des Renntieres (*Rangifer tarandus* L.). Russ. Arkh. Protist. **4**, 43–65 (1925).
4. Dogiel, V. Monographie der Familie Ophryoscolecidae. Arch. Protistenk. **59**, 1–288 (1927).
5. Dogiel, V. La faune d'infusoires habitant l'estomac du buffle et du dromadaire. Ann. parasitol. **6**, 323–338 (1928).
6. Dogiel, V. Eine Notiez über die Infusorien des Renntiermagens. (Russian text, German summary.) Trudy Arkt. Inst. **24**, 143–148 (1935).
7. Dogiel, V. and Fedorowa, T. Ueber den Bau und die Funktion des inneren Skeletts der Ophryoscoleciden. Zool. Anz. **63**, 97–107 (1925).
8. Kofoid, C. A. and MacLennan, R. F. Ciliates from *Bos indicus* Linn. II. A revision of *Diplodinium* Schuberg. U. Calif. (Berkeley) Publ. Zool. **37**, 53–152 (1932).
9. Lubinsky, G. Studies on the evolution of the Ophryoscolecidae (Ciliata : Oligotricha). II. On the origin of the higher Ophryoscolecidae. Can. J. Zool. **35**, 135–140 (1957).
10. Lubinsky, G. Studies on the evolution of the Ophryoscolecidae (Ciliata : Oligotricha). III. Phylogeny of the Ophryoscolecidae based on their comparative morphology. Can. J. Zool. **35**, 141–159 (1957).
11. Lubinsky, G. Ophryoscolecidae (Ciliata : Entodiniomorphida) of reindeer (*Rangifer tarandus* L.) from the Canadian Arctic. I. Entodiniinae. Can. J. Zool. **36** 819–835 (1958).

ANATOMY, HISTOLOGY, AND SECRETIONS OF SALIVARY GLANDS OF THE LARGE MILKWEED BUG, ONCOPELTUS FASCIATUS (DALLAS) (HEMIPTERA: LYGAEIDAE)[1]

JOAN F. BRONSKILL,[2] E. H. SALKELD,[3] AND W. G. FRIEND[3]

Abstract

As in most Heteroptera, the salivary system of *Oncopeltus fasciatus* (Dallas) consists of a pair of trilobed principal glands and a pair of tubular accessory glands with associated ducts; the anatomy and histology are reported in detail. Tests for digestive enzymes demonstrated the presence of amylase, protease, invertase, and lipase. The various lobes of the salivary glands contain different digestive enzymes, a finding that is at variance with some published reports on allied Heteroptera.

Introduction

Though the salivary glands of many hemipterous insects have been studied because of their importance in transmitting virus infection to plants, the anatomy of these glands and the nature of their salivary secretions have not been accurately described in *Oncopeltus fasciatus* (Dallas), one of the common members of this group.

The salivary glands of insects are ectodermal in origin, and have developed as paired glands from the labial segment. In hemipterous insects each salivary gland consists of two major parts, the principal gland and an accessory gland. The size and number of lobes present in each principal and accessory gland vary, though a bilobular principal gland and a unilobular accessory gland are the most usual. Bugnion and Popoff (2) and Dokroscky (5) considered that a bilobed principal gland with a unilobular accessory gland is the primitive condition in Hemiptera.

Southwood (12) has shown that the anatomies of the salivary glands of terrestrial Heteroptera can be correlated more closely with the taxonomic relationships of the species than with the feeding habits or the nature of the salivary secretions. His sketch of the salivary system of *O. fasciatus* indicates that he included the accessory gland as part of the salivary system. He proposed the use of the salivary gland structure in higher classification, and placed *O. fasciatus* in the taxonomic group Pentatomomorpha, characterized by tubular accessory glands with convoluted ducts.

The function of the salivary glands in insects and the nature of the salivary secretions were reviewed by Day and Waterhouse (4). Baptist (1) studied the digestive enzymes present in the salivary glands of 20 species of Hemiptera, and Nuorteva (9) made a similar study on 8 species of wheat-injuring Hemiptera.

[1]Manuscript received July 23, 1958.
Contribution No. 3835, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Entomology Laboratory, Belleville, Ontario.
[3]Crop Insect Section, Entomology Laboratory, Ottawa, Ontario.

This is a report on the anatomy and histology of the salivary glands of *O. fasciatus* together with data on the nature and localization of certain digestive enzymes in the various lobes of these glands. All studies were made on newly molted, feeding adults, reared in the laboratory on milkweed seeds and tap water.

Anatomy of the Salivary System

These studies were made on living material dissected in physiological saline solution, and on material fixed in alcoholic Bouin's and Carnoy's fluids. Toluidine blue O stain was used to differentiate the tissues during the dissections. All drawings were made with the aid of an Abbé-type camera lucida.

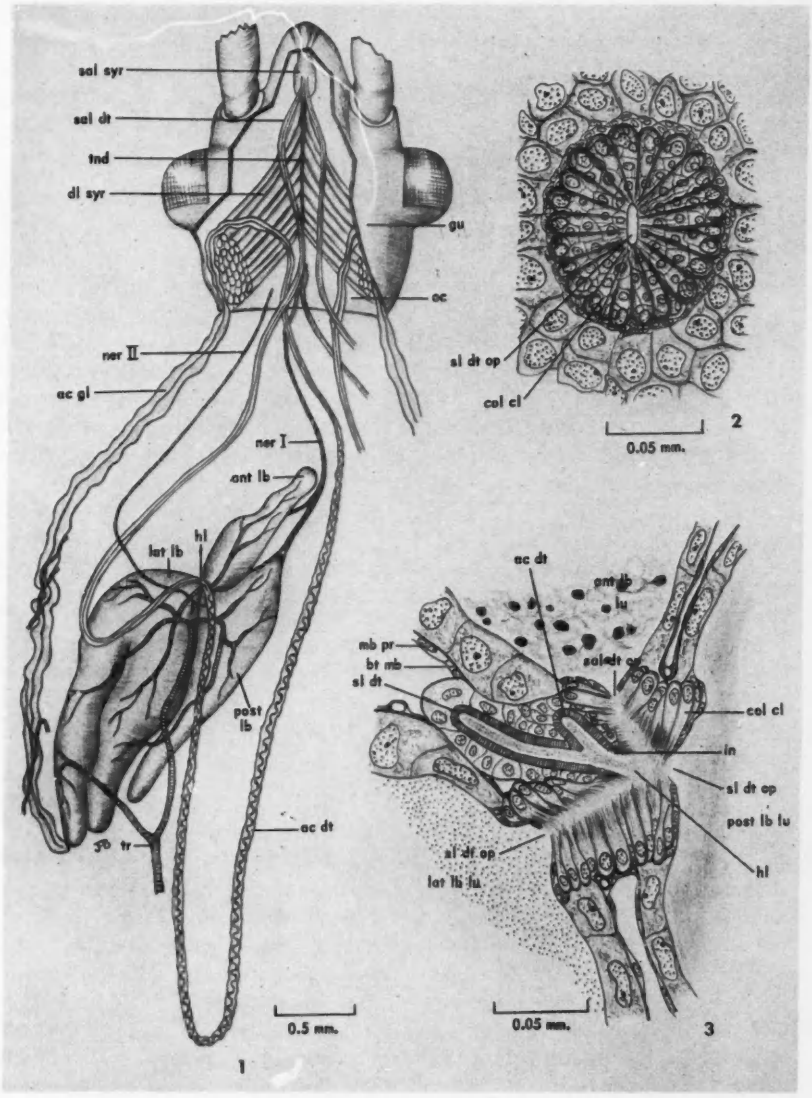
Principal Glands and Ducts

In the thoracic cavity of *O. fasciatus* the paired, trilobed principal glands lie dorsolaterally along the alimentary canal in the region where the esophagus merges into the first stomach. Each gland is about 3 mm long and 1 mm wide and consists of an anterior, a lateral, and a posterior lobe (Fig. 1, *ant lb*, *lat lb*, *post lb*). The lateral lobe is bilobed. The three lobes are joined at the hilus (Fig. 1, *hl*), where the salivary duct emerges from them. The openings of the various lobes into the salivary duct are similar in structure (Figs. 2, 3, *sl dt op*). Each lobe is covered by a network of tracheae and tracheoles, which are branches of the visceral tracheae of the first thoracic spiracle (Fig. 1, *tr*). The salivary glands are partially innervated by the stomodaeal nervous system (11). Each principal gland receives one branch of a paired nerve (Fig. 1, *ner* I) that extends back from the ganglionic mass that lies centrally above the brain. Near the principal gland this nerve branch bifurcates, the main tract going to the anterior end of the posterior lobe and the other to the dorsal side of the anterior lobe. Another nerve (Fig. 1, *ner* II) extends from the laterocaudal side of the subesophageal ganglion and innervates the principal gland, branching just caudad of the hilus and immediately becoming associated with the lateral lobe and the accessory duct.

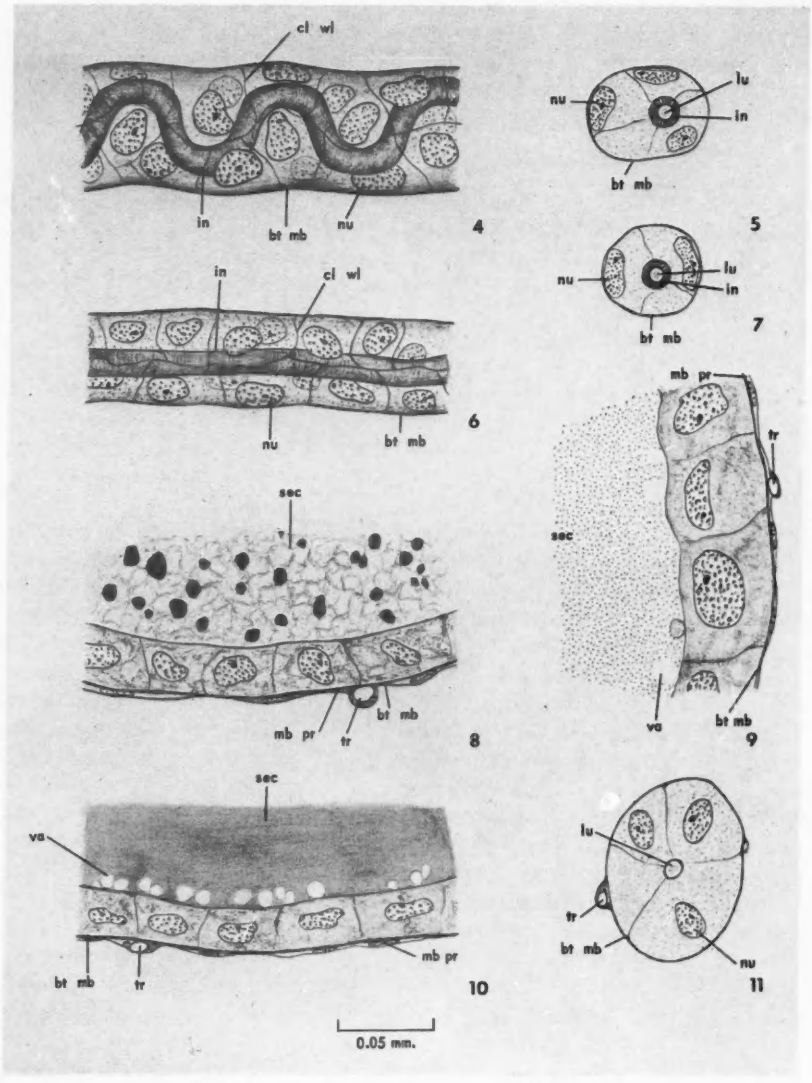
When the salivary duct (Fig. 1, *sal dt*) leaves the hilus it extends back for a short distance between the principal gland and the first stomach. The duct then turns and continues forward to enter the head capsule beneath the dilator muscles of the syringe. As they enter the cylinder of the syringe (Fig. 1, *sal syr*), the paired salivary ducts fuse to form the common salivary duct.

Accessory Glands and Ducts

The tubular accessory gland lies just beneath the principal gland, with its distal end closely associated with the caudal end of the lateral lobe (Fig. 1, *ac gl*, *lat lb*). The accessory gland extends forward in a somewhat convoluted path to the lateral region of the head capsule, where it loops ventrally beneath the dilator muscle of the syringe (*dl syr*), near its origin on the integument, and becomes the accessory duct (*ac dt*). Each accessory duct continues back for a short distance above the salivary duct as a narrow tube, and then enlarges



FIGS. 1–3. *Oncopeltus fasciatus*. 1. Ventral view of a dissection of the right salivary system showing the relative sizes and relationships of the parts. 2. Internal view of the posterior lobe showing the salivary duct opening. Delafield's haematoxylin and eosin. 3. Longitudinal section through the hilus of the principal gland showing association of the salivary and accessory ducts with the principal gland. Delafield's haematoxylin and eosin.



FIGS. 4–11. All figures prepared from material fixed in Helly's fluid, and stained with Delafield's haematoxylin and eosin. 4. Portion of the accessory duct. 5. Cross section of the accessory duct. 6. Portion of the salivary duct. 7. Cross section of the salivary duct. 8. Longitudinal section through part of the anterior lobe. 9. Longitudinal section through part of the lateral lobe. 10. Longitudinal section through part of the posterior lobe. 11. Cross section of the accessory gland.

slightly as its cuticular intima becomes convoluted (Fig. 4). The accessory duct then continues posteriorly along the alimentary canal to the anterior abdominal region, where it loops again and proceeds anteriorly to join the salivary duct at the hilus (Fig. 3). The tubular accessory gland is well tracheated. As noted by Wells (15), the accessory gland in this species is attached to the thoracic gland for the greater part of the length of the latter.

Salivary Syringe

The salivary syringe of *O. fasciatus* has the same mechanism as that of *Anasa tristis* (DeG.) (13) and *Pentatoma* sp. (14). It consists of a cuticular cylinder and pump. The common salivary duct enters the salivary cylinder on its cephaloventral wall. The opening into the salivary pump canal from the salivary cylinder is on the cephalodorsal wall of the latter. Valves that control the movement of the salivary fluid are present in the efferent and afferent openings of the salivary cylinder. A cuticular tendon on which the dilator muscles are inserted is attached to the caudal end of the pump (Fig. 1, *tnd*, *dl syr*). These muscles control the pumping action of the syringe and originate in the gular region of the head capsule, and not on the hypopharyngeal wings as in many other Hemiptera (3).

Histology of the Glands and Their Ducts

Salivary glands preserved in alcoholic Bouin's, Helly's, and Carnoy's fluids were used for histological preparations. This material was dehydrated in ethyl alcohol, cleared in benzol, embedded in Tissuemat (melting point, 54–56° C),[4] sectioned at 7 μ, and stained in Delafield's haematoxylin and eosin, in Mallory's phosphotungstic acid haematoxylin, and by the Bodian technique.

Principal Glands and Ducts

Baptist (1) concluded that the cellular structures of the principal glands of Heteroptera vary according to the type of food consumed. Each salivary gland of carnivorous Heteroptera has a very small lumen and long columnar cells with secretory products stored as zymogenic granules within the cytoplasm; in herbivorous Heteroptera each gland has a large lumen and the glandular epithelium consists of a layer of short secretory cells. The principal glands of *O. fasciatus* are similar to those of the herbivorous species described by Baptist. The wall of each lobe consists of a layer of simple cuboidal epithelium mounted on a basement membrane. Surrounding the basement membrane is a layer of simple squamous epithelium, the membrana propria (Figs. 8, 9, 10, *bt mb*, *mb pr*). The cells in both lobules of the lateral lobe appear to be slightly taller than those of the other two lobes (Fig. 9). In sections stained with Delafield's haematoxylin and eosin the nuclei and cytoplasm of the three lobes are extremely basophilic. In the silver-impregnated tissue the cytoplasm in the cells of the anterior and lateral lobes, like

[4]Fisher Scientific Co., Toronto 8, Ontario.

that in the accessory gland, contained many darkly staining granules. The opening of each lobe into the salivary duct is surrounded by several layers of columnar uninuclear cells (Figs. 2, 3, *col cl*).

In all the lobes the secretion is of the merocrine type and, as it is precipitated by most fixatives, appears to be proteinous. After staining with Delafield's haematoxylin and eosin, the secretion in the anterior lobe appears as a reddish-brown reticulum with bluish-brown granules throughout it (Fig. 8, *sec*). The secretion of the lateral lobe appears as fine, faintly eosinophilic granules, and that of the posterior lobe as an intensely eosinophilic, gelatinous mass (Figs. 9, 10, *sec*). A few colorless vacuoles and basophilic globules are present at the peripheries of the secretions in the lateral and posterior lobes. Mallory's phosphotungstic acid haematoxylin stain also differentiates the secretion in each lobe of the principal gland: the anterior lobe shows a peach-to-purple reticulum, the lateral lobe fine peach-colored granules, and the posterior lobe a light, peach-colored, gelatinous mass. These observations indicate chemical and physical differences in the secretions of the various lobes.

The salivary duct consists of simple cuboidal uni- or bi-nucleated cells. Its inner cuticular intima is thickly striated to form the wall of the lumen (Fig. 7, *in*). This wall, however, is not convoluted like that of the accessory duct. In material stained with Mallory's phosphotungstic acid haematoxylin or with Delafield's haematoxylin and eosin, the striated cuticular intima stains a deep pink. A noncellular basement membrane (Fig. 7, *bt mb*) forms the outer border of the duct. The cytoplasm and nuclei of the duct are slightly less basophilic than the cytoplasm and nuclei of the principal gland cells. The fine granular secretion in the lumen of the duct appears faintly basophilic.

Accessory Glands and Ducts

The accessory gland is formed by cuboidal glandular cells surrounding a small lumen that is similar in diameter to that of the accessory duct (Figs. 5, 11, *lu*). In any cross section of the gland four such cells are usually visible. A thin, taenidia-like border forms the inner wall of the gland. Each cell contains one or two large, oval nuclei with fine, granular chromatin. The cytoplasm of these cells is somewhat less basophilic than that of the cells in the principal gland, and sections stained by the Bodian technique show black granules in the cytoplasm. The fine, granular secretion of the accessory gland appears faintly basophilic in Delafield's haematoxylin and eosin preparations, and fawn-colored in material stained with Mallory's phosphotungstic acid haematoxylin.

The accessory duct, like the salivary duct, consists of simple cuboidal uni- or bi-nucleated epithelial cells (Figs. 4, 5). The inner cuticular intima of the duct is thickly striated. This striated cuticular border is regularly convoluted for the greater part of its length, giving the duct the appearance of a convoluted tube (Fig. 4, *in*). Both Mallory's phosphotungstic acid haematoxylin, and Delafield's haematoxylin and eosin stain the striated cuticular intima deep pink. The cytoplasm of the duct cells is similar in

basophilia to the cytoplasm of the accessory gland and salivary duct cells. The nuclei appear similar in nature to those of the salivary duct cells. The fine, granular secretion in the lumen of the duct stains similarly to the secretion in the lumen of the accessory gland.

Digestive Enzymes in the Salivary System

Methods

Preliminary tests at pH 4.2, 5.3, 6.3, and 7.4 showed that pH 6.3 was optimum for enzyme reactions with the various lobes. The procedures used to demonstrate the presence of enzymes were as follows:

1. *Amylase.*—Lobes from the salivary glands of 10 insects were separated and ground with a drop of 0.2 *M* phosphate buffer, pH 6.3, in a micromortar. A micropipette was used to transfer small drops of this brei to a glass slide thinly coated with a starch agar film (10). The slide was then incubated in a saturated atmosphere at 37° C for 1½ to 2 hours. Human saliva and drops of the buffer solution were used as controls. After incubation the slide was rinsed in distilled water and stained with dilute iodine.

2. *Protease.*—Lobes from the salivary glands of 10 insects were separated and ground in a drop of buffer solution, pH 6.3, as described for the amylase test. The presence of protease was determined by the gelatin slide method of Pickford and Dorris (10). A 2% protease solution[5] and drops of the buffer solution were used as controls.

3. *Invertase.*—Lobes from the salivary glands of 10 insects were separated, ground, and placed in microtubes with a drop of buffer solution, pH 6.3. A 5% invertase solution[5] was used as a control. A drop of 5% sucrose was put in each tube as the substrate. A drop of toluol was finally added to each tube, and the tubes were incubated at 37° C for 1 hour. After incubation, a drop of Benedict's solution was added to each tube. The tubes were then boiled for a few minutes.

4. *Lipase.*—Salivary glands from eight insects were dissected in cold acetone and fixed in fresh cold acetone for 24 hours. They were then embedded in paraffin, sectioned, and treated for the determination of lipase by the Tween 60 method of Gomori (6).

Results

Strong amylase activity occurred in the lateral lobe brei (Table I), as shown by the complete dissolution of the starch film; slight activity, indicated by a reddening of the starch film around the edges of the drops, occurred in the posterior lobe brei. Neither of the other lobes showed any amylase activity. Slight protease activity, resulting in the dissolution of the gelatin film at the droplet edges, was found in the posterior lobe brei at pH 6.3, 5.3, and 4.2. No activity was detected in the remaining three lobes, although a darkly stained area around the droplets of the accessory gland suggested possible weak activity.

[5]Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.

TABLE I

OCCURRENCE OF DIGESTIVE ENZYMES IN THE VARIOUS LOBES OF THE SALIVARY GLAND of *O. fasciatus**

Enzyme	Lobe			
	Anterior	Lateral	Posterior	Accessory
Amylase	−	++	+	−
Protease	−	−	++	+
Invertase	+	++	+	−
Lipase	−	−	++	−

*−, no activity; +, slight activity; ++, high activity.

A strong positive reaction to Benedict's test indicated invertase activity in the lateral lobe; weaker positive reactions occurred with the anterior and posterior lobes but not with the accessory lobe. Lipase, indicated in the Tween 60 method by the presence of a golden-brown color at its site of activity, was found in large amounts in the lumen and in lesser amounts along the inner cell border of the posterior lobe. This enzyme was not detected in the other three lobes.

General Discussion

The results given herein add to the information on the salivary glands of *O. fasciatus*, and are in part at variance with speculations advanced by some previous authors on the secretions of the salivary glands of Hemiptera in general.

This detailed study of the accessory gland permits an enlargement and clarification of the view expressed by Hood (7) in 1937: that the convoluted duct is a part of the principal gland that runs from the hilus of the lobes of the principal gland into the head region. He failed to observe that the convoluted duct (accessory duct) is actually connected with the accessory gland in the head region, probably because he failed to note the presence of the accessory gland. The authors think that the accessory gland and duct must be considered a functional part of the salivary system in *O. fasciatus* because of its intimate anatomical relationships to the principal gland, though enzyme tests on it revealed only a slight protease activity. Southwood (12) also considered the accessory gland to be part of the salivary system in this insect. The accessory gland has been considered as part of the salivary system in most of the studies conducted on other Hemiptera.

The results of the histochemical and enzyme tests do not confirm generalizations by Baptist (1) and Day and Waterhouse (4). Baptist (1), who studied 20 species of Heteroptera, found that not more than two enzymes were present in any salivary gland system. In 10 species, in which he separated the glands into anterior and posterior portions and tested the accessory gland separately, he found the same enzymes in both the anterior and posterior portions but was unable to find any trace of an enzyme in the accessory gland.

On the basis of these findings he concluded, "All the information obtained in this work is strongly against the idea that the various lobes of Hemipterous salivary glands produce widely different chemical substances, each with a special function". Day and Waterhouse (4), in their review on the function of the salivary glands in insects, stated, "In spite of the complex structure of the glands of Hemiptera there is no evidence that different materials are produced by the various lobes". It is noteworthy, therefore, that the salivary glands of *O. fasciatus* were found to contain four enzymes; that different enzymes were found in different lobes; and that histochemical tests indicated chemical and physical differences in the secretions of the various lobes.

Baptist (1) further stated that the enzymes present in the 20 species of Heteroptera studied were all concerned with the digestion of that particular component of the food that was present in the greatest proportion: herbivorous forms have invertase or amylase, or both, and carnivorous forms usually have protease or lipase, or both. Mr. John K. Campbell of the Ottawa laboratory has recently noted that *O. fasciatus*, which is normally phytophagous though it is known to eat its own eggs, will feed on newly molted adults of its own species. *O. fasciatus*, therefore, would derive benefit from a full complement of digestive enzymes since it is not strictly phytophagous.

Nuorteva (9) found that *Lygus rugulipennis* Popp., another species of Heteroptera, lacks proteases in the adult salivary glands but possesses them in the nymphal state. Kretovich *et al.* (8) observed that proteases appear when *Eurygaster integriceps* Put. changes from feeding on the growing, green parts of the wheat plant to the ripening kernels. The last two observations indicate that the enzyme complement is not constant, and consequently studies of this type should be conducted on insects of known age and physiological condition.

References

1. Baptist, B. A. The morphology and physiology of the salivary glands of Hemiptera: Heteroptera. Quart. J. Microscop. Sci. **83**, 91–139 (1941).
2. Bugnion, E. and Popoff, N. L'appareil salivaire des Hémiptères. II. Arch. Anat. Micr. **11**, 435–456 (1910).
3. Butt, F. H. Comparative study of mouth parts of representative Hemiptera–Homoptera. Cornell Univ. Agr. Expt. Sta. Mem. 254 (1943).
4. Day, M. F. and Waterhouse, D. F. Functions of the alimentary system. *In* Insect physiology. *Edited by* K. D. Roeder. John Wiley and Sons, Inc., New York, N.Y. 1953. pp. 299–310.
5. Dokroscky, I. D. Morphological and cytological studies on the salivary glands and alimentary tract of *Cicadula senotata* (Fallen), the carrier of aster yellow virus. Hemiptera. Contrib. Boyce Thompson Inst. **3**, 39–58 (1931).
6. Gomori, G. The study of enzymes in tissue sections. Am. J. Clin. Pathol. **16**, 347–352 (1946).
7. Hood, C. W. The anatomy of the digestive system of *Oncopeltus fasciatus* Dall. (Heteroptera–Lygaeidae). Ohio J. Sci. **37**, 151–160 (1937).
8. Kretovich, V. L., Bundel, A. A., and Pshenova, K. V. Mechanism of wheat injury by *Eurygaster integriceps*. Compt. rend. acad. sci. U.R.S.S. (N.S.), **39**, 31–33 (1943). Rev. Appl. Ent. **32**, 160–161 (1944).
9. Nuorteva, P. Studies on the salivary enzymes of some bugs injuring wheat kernels. Ann. Entomol. Fenn. **20**, 102–124 (1954).
10. Pickford, G. E. and Dorris, F. Micro-methods for the detection of proteases and amylases. Science, **80**, 317–319 (1934).
11. Snodgrass, R. E. Principles of insect morphology. McGraw-Hill Book Co., Inc., New York, N.Y. 1935.

12. SOUTHWOOD, T. R. E. The morphology of the salivary glands of terrestrial Heteroptera (Geocorisae) and its bearing on classification. Tijdschr. Entomol. **98**, 77–84 (1955).
13. TOWER, D. G. The mechanism of the mouth parts of the squash bug, *Anasa tristis* DeG. Psyche, **21**, 99–108 (1914).
14. WEBER, H. Biologie der Hemipteren. Springer-Verlag, Berlin. 1930.
15. WELLS, M. J. The thoracic glands of Hemiptera Heteroptera. Quart. J. Microscop. Sci. **95**, 231–244 (1954).

ABBREVIATIONS USED IN FIGURES

ac dt, accessory duct; *ac gl*, accessory gland; *ant lb*, anterior lobe of the principal gland; *bt mb*, basement membrane; *cl wl*, cell wall; *col cl*, columnar cell; *dl syr*, dilator muscle of the syringe; *gu*, gula; *hl*, hilus of the principal gland; *hyp wg*, hypopharyngeal wing; *in*, intima; *lat lb*, lateral lobe of the principal gland; *lu*, lumen; *mb pr*, membrana propria; *ner* I, nerve of the stomodaeal nervous system; *ner* II, nerve of the central nervous system; *nu*, nucleus; *oc*, occiput; *post lb*, posterior lobe of the principal gland; *sal dt*, salivary duct; *sal syr*, salivary syringe; *sec*, secretion of the principal gland; *sl dt op*, salivary duct opening; *tnd*, cuticular tendon to which the dilator muscles of the syringe are inserted; *tr*, trachea; *va*, vacuole.

THE INHERITANCE OF LITTER SIZE, BODY WEIGHT, AND VARIABILITY, IN A CROSS BETWEEN TWO INBRED STRAINS OF MICE[1]

L. BUTLER

Abstract

Crosses were made between the two inbred lines, C57BL and BALB, and the effects of maternity and genotype on litter size, survival, 30- and 60-day weights, and their variances were assessed. The maternal effect of BALB reduced litter size by .78 to 1.91 when compared with reciprocal crosses of identical genotype. No maternal effect was found for C57; neither did the genotype of the young have any effect on the litter size borne by mothers of this strain. There was a significant increase ($2.26 \pm .67$) in litter size when BALB females produced hybrid instead of inbred young. This was not the result of hybrid males being more prolific, because BALB males mated to non-BALB females produced the largest litters in this experiment. The cross $F_1 \times F_1$ produced the largest mean litter size. A change in ration affected the litter size when BALB was the mother, but not when C57 was the mother. Fox chow plus linseed produced more young than any of the other three diets.

There were no significant differences in the weights of males of any of the generations at 30 days, but the F_1 females borne in C57 mothers were larger than the females of other generations, and it appears that the C57 female exerts a maternal effect which causes the female young to mature earlier. The data indicate that the mother exerts the greatest influence on 30-day weights, the genotype of the young having little or no effect. The F_1 female has the greatest maternal effect: she increases the 30-day weight by 10–20%.

At 60 days the F_1 mouse was 15% larger than the corresponding inbred borne in the same female. The mean weights of the F_1 and F_2 were the same, but the backcrosses were 10% lighter.

The variance at 30 days was larger than the corresponding variance at 60 days, and 90% of the variance was in the between-litters component. At 30 days the largest variances were found in the P_1, F_1, and B.C. generations, while the smallest was found in the F_2 indicating that much of the variability was the result of the maternal environment. At 60 days the variances for all generations were essentially the same.

MacArthur's large and small strains, which had been produced by 30 generations of selection with only moderate inbreeding, were carried through 20 generations of brother × sister mating. This inbreeding brought about a decrease in the weight of the large line, and an increase in the weights of the small strain. The former may be attributed to the loss of the heterozygotic effect on size, while the latter may be the result of natural counterselection; the smallest mice had few or no young.

Introduction

The study of the inheritance of body size in animals is complicated by the fact that it is extremely difficult to assess the relative effects of heredity and environment. When a cross is made between outbred animals it is difficult to assign a value to the parental heterozygosity. On the other hand, if inbred animals are used for the parental stock, it is hard to assess the "inbreeding depression" in the P_1. This experiment was undertaken to evaluate inbreeding depression and to contrast with a former study in which outbred stocks were used. The two inbred strains used in this experiment have approximately the same body size and growth rate, but since they differ in the coat-color genes, black vs. brown, agouti vs. non-agouti, colored vs.

[1]Manuscript received July 16, 1958.
Contribution from the Department of Zoology, University of Toronto, Toronto, Ontario.

albino, it is highly probable that they also differ in genes for body size. Furthermore, when several generations are raised, it is possible to estimate the inbreeding depression by comparison of F_1 with parents, and F_1 with F_2. The two inbred lines and the cross-bred generations derived from them all contain the same genes: they differ only in degree of heterozygosity or in interactions between non-allelic genes.

Supplementary information on the manner in which inbreeding affects body size was obtained from 12 generations of brother $\times$ sister mating using the large and small strains selected by MacArthur (4).

Material and Methods

The stocks used in this study are:

MacArthur's large strain selected for large body-size but maintained by mating the largest individuals without any conscious attempt at inbreeding.

MacArthur's small strain selected for small body-size and not inbred.

C57BL, the standard strain inbred by means of brother $\times$ sister mating for 61 generations. The genetic constitution is *aa BB CC*, the fecundity fair with an average litter size 5.8 young. The average weight of males at 60 days is 18.5 g while that of the females is 15.5 g. These weights are less than those reported by Searl (7) and are probably the result of a difference in diet rather than a sub-line difference.

BALB, a standard line inbred for 92 generations. It has the genetic constitution *AA bb cc*, the fecundity is fair but not as good as C57. The average litter size at birth is 4.4 young and the 60-day weights 18.6 g for males and 15.9 g for females.

The breeding cage consists of one male and five to eight females. When a female becomes noticeably pregnant she is isolated and kept in a separate cage with her litter until the young are 30 days old. This prevents the mother from being bred immediately after parturition, and thus keeps her from being pregnant at the time she is nursing her young. The ration consists of standard fox chow fed ad libitum, and of supplementary feedings of lettuce. While this ration gives good results with outbred stock there is some evidence that more complex rations will give better growth and reproduction in some inbred strains. Whether this is because it is necessary to pamper the inbred strains, or because each inbred has different requirements for the various nutrients as shown by the riboflavin research of Fenton and Cowgill (3), has not been ascertained.

The young are weighed at 30 days, ear punched, and their coat-color phenotype is noted. The mother is returned to the breeding cage, and the young are left in this cage until the final weighing at 60 days of age. This procedure is modified for hybrid young or large litters by separating the two sexes and using two cages instead of one.

Litter Size

The pertinent data on the number of young per litter are given in Tables I and II. The evaluation of maternal and genotypic effects by these data is made possible; comparisons between the results of reciprocal crosses reveal the effect of the maternal environment, while comparisons of the litter sizes

TABLE I

NUMBER OF YOUNG BORN AND RAISED PER LITTER IN EACH GENERATION

Parents		No. litters	No. males	No. females	Mean litter size		Die between birth and 60 days
Female	Male				Birth	60 days	
C57	C57	35	96	84	5.80±.29	5.14±.30	.66
BALB	BALB	37	59	73	4.41±.36	3.57±.30	.84
BALB	C57	18	43	51	6.67± 57	5.17±.39	1.50
C57	BALB	20	53	56	6.05±.22	5.35±.33	.70
F_1	F_1	28	97	98	7.50±.38	6.89±.35	.61
F_1	F_1	22	66	83	7.32±.37	6.68±.38	.64
F_1	F_1	34	96	102	7.47±.39	6.93±.31	.54
C57	F_1	16	38	52	6.00±.40	5.60±.42	.40
F_1	C57	18	54	50	6.50±.42	6.17±.44	.33
BALB	F_1	10	27	23	6.10±.60	5.10±.57	1.00
F_1	BALB	27	80	86	6.88±.31	6.22±.28	.66
B.C.	BALB	16	44	55	7.67±.20	6.19±.21	1.48
BALB	B.C.	29	71	67	5.76±.40	4.75±.27	1.01
B.C.	C57	13	37	39	6.23±.38	5.85±.28	.38
C57	B.C.	30	83	80	6.23±.21	5.10±.31	1.13

TABLE II

DISTRIBUTION OF LITTER SIZES IN THE VARIOUS GENERATIONS

Parents														Total
♀	♂	2	3	4	5	6	7	8	9	10	11	12	13	litter
C57	C57		4	3	10	5	7	4	2					35
BALB	BALB	5	5	13	4	5	4	1						37
C57	BALB			1	5	7	6	1						20
BALB	C57		1	2	5	2	2		3	2	1			18
F_1	F_1			1	9	25	12	13	9	6	3	1	1	80
F_1	C57		1	1	2	6	4	1	2	1				18
C57	F_1			3	5	2	1	5						16
BALB	F_1	1			2	3	2	1	1					10
F_1	BALB				5	9	4	5	2	1	1			27
B.C.	BALB			1	1	1	4	4	3	1	1			16
BALB	B.C.	2	1	2	12	2	6	2	1				1	29
B.C.	C57		1	1	4	2	1	2	1	1				13
C57	B.C.		1		5	13	8	2	1					30

of different genotypes in the same maternal environment allow one to assess the genotypic effects. The over-all picture shows that litter size increases from the P_1 to the F_1, reaches its maximum in the F_2, and then declines again in the backcrosses.

The maternal influence on litter size is best seen in the two sets of reciprocal backcrosses. When the BALB strain is used as the female parent the litter contains 0.78 less young than in the reciprocal cross where embryos of the same genotype are borne in F_1 females. A similar result is obtained when BALB females are mated to backcross males: here the litter size is 1.91 smaller than in the reciprocal cross. In the production of F_1 young the difference is in the opposite direction, the BALB females producing litters which are $.62 \pm .61$ larger than those of the same genotype produced by C57 females. Although this difference is not statistically significant, it may indicate that the C57 females are normally producing litters of a size which is near their inherent capacity whereas the BALB females, because of poor implantation or early deaths of the embryos, have reserve capacity and can be stimulated to produce more young when the appropriate male is used.

The maternal effects of C57 can be assessed from a similar set of crosses. In the backcross the litters are smaller by $.5 \pm .58$ young when C57 is the mother than they are in the reciprocal cross when C57 is the father. In the cross B.C.$\times$C57 there is no difference between reciprocals. Thus it appears that C57 does not exert any significant maternal effect on litter size.

The effect of the genotype of the young on the number of embryos that become implanted and carried to full term is best illustrated by comparing the size of litter that each P_1 produces when carrying young of its own genotype with the litter sizes produced when it is carrying young of other genotypes. Comparing the average number of young produced per litter by C57 females, Table I, we find that all litters are of approximately the same size irrespective of the genotype of the young; the mean number of young per litter in each generation is 5.8, 6.05, 6.0, and 6.23 for P_1, F_1, F_2, and B. C. respectively. It is true that the litter size of the outbred young is in all three cases larger than the mean litter size of the inbred young, but even the largest difference of $.43 \pm .36$ does not approach significance.

The genotype of the young does affect the size of the litter when BALB is used as the female parent. When BALB females gestated F_1 young, the litter size increased $2.26 \pm .67$ over the mean litter size obtained when the female bore young of the BALB genotype. This 50% increase in litter size shows the effect of genotype on either implantation or survival in the uterus. When BALB females gestate backcross young, the litter sizes are $1.69 \pm .70$ and $1.35 \pm .56$ larger than when they gestate BALB young. These three significant increases cannot be the result of the BALB males being less prolific, as is revealed by the two following points. First, the F_1 females mated with BALB males produce larger litters than the same females crossed with C57 males. Secondly, backcross females mated to BALB males produce the largest litters in this series of crosses. The increased litter size of BALB

females when bred to other than BALB males may be the result of such things as: outcross sperm are more successful than sperm of the inbred strain; the mating response to the different type male causes more eggs to be shed; or the genotype of the hybrid embryos enables more to survive to parturition than in the case of embryos with the inbred genotype. This latter seems the more plausible reason for the increase; it appears that litters of BALB young are smaller than the genetic potential size because they are not adapted to the environment of the BALB uterus. Hybrid embryos with the homeostatic effect of their heterozygosity are able to adapt themselves to this uterus and therefore more of them reach full term.

When the F_1 female is used as the mother, the litter sizes are 7.5 for the F_2 generation, and 6.5 and 6.88 for the two backcrosses. The backcross values are not significantly different, either C57 or BALB males being equally effective for this purpose. The F_1 males, on the other hand, are definitely superior, producing significantly more young. The increased size of litter in this case cannot be the result of heterozygosity alone, because both F_2 and backcross young should be heterozygous for 50% of the genes which are segregating in this cross; therefore the difference must be the result of the increased vitality of the F_1 males and their sperm.

The mean litter sizes given in Table I do not tell the whole story because the distributions they describe are not normal. The frequency distribution of number of young per litter is given in Table II. From this table it is seen that C57 produced litters with a modal class of five young, and that the remainder of the litter-size classes, which range from three to nine young, occur with equal frequency. BALB, on the other hand, has a modal class of four and the rest of the litters are evenly divided among the size classes two to seven. The difference between the maternal effects of the two parental lines would account for this real difference between the two distributions, for the main component of difference is the frequent occurrence of litters of two young in the BALB line. If the distribution of litter size of the BALB inbreds is transformed by the addition of one young to each litter, then the BALB and C57 distributions are no longer significantly different but instead are almost indentical. Thus the differences in litter size between the two parental strains may be entirely the result of the maternal effect of BALB.

When a BALB male is mated to a C57 female the distribution becomes more concentrated, 90% of the litters consisting of five, six, or seven young. In the reciprocal cross the BALB females produce almost equal numbers of litters of sizes 3 to 11. So that while there is no significant difference in the means of these reciprocal crosses, their composition is entirely different. In the first cross the increased litter size of the F_1 is brought about by shifting the mode upwards and decreasing the number in the tails of the distribution, whereas in the other case the increase is brought about by eliminating litters of 2, and replacing them with litters of 9, 10, and 11, large litter sizes which do not occur when BALB is mated with BALB.

The C57 females are evidently working near capacity when they are producing inbred young; as a result of this the response elicited by the F_1 genotype is a slight raising of the modal class and a greater uniformity of litter size. The backcross young show essentially the same distribution of litter size as that exhibited by the inbred parents. The largest litter sizes occur when F_1's are mated together, which is similar to the result obtained by Chai (2). The mating $F_1 \times F_1$ gives no litters with a small number of young, but litters with large numbers of young do occur making the distribution positively skewed with a mean of 7.5 and a mode of 6.0 young per litter. The uniform, though heterozygous, genotype of the mother has not produced a uniformity of litter size, but instead has produced more variability. This variability is not the result of one or two females producing large litters; the between females, and the within female variances are equal.

The backcrosses also have few small litters and a wide range of litter sizes. Backcross males mated with BALB females produce litters which range in size from 2 to 13. The mice in the litter of 13 were very small at birth and unthrifty during the nursing period: seven of the young died in the first 20 days, and at 60 days of age the surviving young were 20% below the weight of young of similar constitution from average-sized litters.

These data demonstrate that BALB and C57 females produce litters of approximately equal size when the genotypes of the young are the same, or even when they are both served by the same type of hybrid male. The only real difference lies in the apparently higher mortality of embryos when BALB females gestate BALB young. To find out if this has a nutritional basis, the two strains were fed on four different diets. These diets were: (1) the standard fox chow diet used throughout this experiment, (2) fox chow plus linseed, (3) rabbit chow, (4) rabbit chow plus linseed. The simple analysis of these diets was:

	Fox chow	Rabbit chow	Linseed
Crude protein	20.0	17.0	22.6
Fat	3.0	2.5	33.7
Crude fiber	5.0	12.0	7.1

These rations give four levels of protein, four levels of fiber, and two levels of fat. The unsaturated fats of linseed are reputed to have a beneficial effect on reproduction, and lactation. Twenty C57 and 28 BALB adult female mice were kept on these diets for 6 months, and the litter size and total young produced by each female was recorded. The data are given in Table III. Examination of this table reveals that there are no significant differences between strains on the same ration. The lone significant difference occurs when rabbit chow is fed. On this diet the C57's produce their largest litters, whereas BALB's produce their smallest, the difference between the two strains being $1.21 \pm .59$. It is interesting to note that the BALB mice on all rations have larger litters than in the main experiment (Table I). The reason for this increase probably lies either in the known effect of season on litter size or the fact that the supplementary feeding of lettuce and spinach was

TABLE III

THE EFFECT OF DIFFERENT RATIONS ON THE PRODUCTION OF YOUNG

		Ration			
	Strain	Fox chow	Fox chow and linseed	Rabbit chow	Rabbit chow and linseed
Litter size	C57	5.71±.49	5.81±.50	5.83±.18	5.41±.49
	BALB	5.80±.56	5.87±.42	4.62±.56	5.37±.87
Young per female	C57	16.0±2.34	23.2±1.89	11.7±0.41	23.0±1.58
	BALB	18.1±2.58	20.2±1.82	12.3±3.20	14.3±1.11
Mean loss per litter before 60 days	C57	1.30	0.82	2.50	2.10
	BALB	2.37	2.04	3.38	1.64

increased from once every 2 weeks to twice a week. The results of this experiment confirm the general conclusions, which we have arrived at above, namely, that C57 is producing near its capacity, and that changing the rations fed to the female or changing the genotype of the young will have little effect on litter size. In the BALB strain, on the other hand, we have already seen that the genotype of the young affects the litter size; we now find that a change in ration will also change the litter size.

The second part of Table III gives the mean number of young per female for each of the four rations. Unlike litter size, the total number of young does vary between the different rations. C57 females produce the most young on diets which contain linseed; the difference between a diet of fox chow alone and fox chow plus linseed is 7.2±3.01 young, while the addition of linseed to the rabbit chow produces 11.3±1.63 additional young. BALB females show similar differences in the number of young produced; here again the diet of fox chow plus linseed produces the most young, but unlike C57, the addition of linseed to the rabbit chow diet does not bring about a significant increase in the number of young born. For both strains, rabbit chow alone constitutes the poorest diet, while fox chow plus linseed gives the best production.

Turning to the number of young raised from each mating we find that the rate of survival is not the same in all cases. Survival to 30 days depends on vitality of the young at birth, and the milk available during the nursing period. Survival to 60 days is also dependent upon their growth and health in the first 30 days of life, and, in addition, upon how well they adapt themselves to the changeover from nursing to solid food. The last column of Table I lists the average number of young per litter that die between birth and the 60th day. Grouping these figures by maternal origin we find that C57 loses an average of .72 young while BALB loses 1.1 young, F_1 .50 young, and B.C. mothers lose .93 young. These figures indicate that the F_1 females are the best mothers. The difference in young lost by the two inbred strains is barely significant but the consistently higher losses of the BALB females indicate

that they are the poorest mothers. Since most of the losses occur in the nursing period, one concludes that BALB is a poorer milk producer than C57. While many of the differences are small and the results involving B.C. mice are somewhat varied, the main conclusion to be drawn is that losses in the first 60 days of life depend on the constitution of the mother rather than on the genotype of the young.

The losses in the experiment involving four different rations are given in Table III, and a comparison with Table I shows that these losses are much higher. In the experiment with rations the young are handled and weighed each day which probably accounts for these higher rates of loss. C57 has less juvenile mortality when fed on rations containing fox chow, whereas BALB has the least mortality on the ration of rabbit chow and linseed. This small experiment is not conclusive but it does indicate that different genetic strains do not give the same response to changes in food, and that the differential juvenile mortality in these two stocks could be reversed by choosing the appropriate ration.

Analysis of the Differences in Weight at 30 Days

The weight attained at 30 days has been used by MacArthur (5) and others as a criterion of early growth. It is difficult to pick the weight on any particular day as comparable on a biological scale because of the differences between individual baby mice in the beginning and ending of weaning, and in the way various mice adapt to a diet of solids. Some mice begin to take solid food at 15 days of age, whereas others are reluctant to wean at 30 days, and are still trying to nurse at 35 days. In general, the average mouse should be weaned and adapted to solid food by the time that it is 30 days old. A plateau in the growth curve occurs from the 15th to 22nd day, and probably reflects the effects of the weaning period. This should be over, and the weight beginning to increase again before the 30-day weight is taken. Thus the 30-day weight is not only a measure of growth rate but also of the speed of weaning and adaptability to the laboratory diet.

The differences in the growth rate of the two parental strains up to this time are negligible. It is true that BALB females are larger than C57 females at 30 days, and that in the 15–20-day period the growth of C57 is checked more strongly than in BALB, but differences in litter mortality make it almost impossible to state whether these differences are real, or interaction effects brought about by competition for a restricted milk supply.

At 30 days the BALB males are $0.7 \pm .49$ larger, and the BALB females are $1.4 \pm .39$ larger than the corresponding C57 mice. Male and female BALB at this age are both the same weight, whereas the C57 females are $0.9 \pm .38$ lighter than their brothers.

The males of the P_1, F_1, and B.C. generations produced by BALB females are almost indentical in weight, there being only 0.4 g difference between the largest and the smallest of the mean male weights for each of these generations. The C57 also produces males which show no significant differences

between the mean weights of each of the three generations at this age, although the difference here, $1.0 \pm .53$, is much greater than in the case of males borne in BALB females. Thus the 30-day weights indicate that there is either no segregation of genes affecting weight at this time, or else the interaction of genotype, milk supply, and weaning setback are such that they cancel out any differences.

The females of the P_1, F_1, and B.C. generations gestated by BALB females are almost identical, whereas the F_1 females produced by C57 mothers are significantly ($1.1 \pm .42$) larger than either the inbred or B.C. females. While all the data are not consistent, there is a suggestion that C57 females exert a maternal effect on their female offspring which causes them to begin to differ from their brothers at an earlier age than do the female offspring of BALB mothers. In the backcross, when C57 is the female, she produces males which are 1.0 larger than their sisters, whereas in the reciprocal cross, male and female are the same weight. In the backcross involving the BALB strain, there are differences of 1.0 and 1.5 between male and female offspring.

In $B.C._2$, the parental strains are crossed with backcross mice picked at random. Table IV shows that while there are differences between reciprocal crosses involving C57, none of the differences are significant. In the crosses to the BALB strain, the B.C. females produce the largest young, the difference between the mean weights of male young from the reciprocal crosses is significant (1.5 ± 0.54), indicating that the BALB female is a poorer environment for the young.

The data presented above indicate that the mother, presumably through her milk supply, exerts the greatest influence on 30-day weights, the genotype of the young having little or no effect. This point may be further emphasized by comparison of the results of the reciprocal backcrosses. In the cross $F_1 \times$ C57 the males are $1.4 \pm .56$ larger and the females $2.4 \pm .35$ larger than in the reciprocal cross where C57 is the mother. In the cross $F_1 \times$ BALB the males are $.8 \pm .68$ larger and the females $1.3 \pm .53$ larger when the F_1 female is the mother. Since genotypes are identical, these increases represent the maternal effect of the F_1 female whose superior environment brings about a 10–20% increase in 30-day weights over those of the inbreds.

The F_2 males are 1.0–1.8 g larger than the P_1's or F_1's whereas the females show less difference. This increase in size is attributable to the better environment, both prenatal and postnatal, supplied by the F_1 females. Thus it appears that the only differences observed at this age are environmental and not genetic.

Analysis of the Differences in Weight at 60 Days

By the time the mice are 60 days old they are mature and their growth rate has slowed down. The mean weights by sexes for each generation are given in Table IV. This table reveals that the parental differences are not significant, BALB mice being less than half a gram larger than the C57 ones. In the F_1, reciprocal crosses show no significant differences although the

TABLE IV

WEIGHTS AND VARIANCES FOR FIVE GENERATIONS OF MICE

		Offspring									
		Males					Females				
		No.	Mean		Variance		No.	Mean		Variance	
Gen.	Parents ♀ × ♂		30 days	60 days	30 days	60 days		30 days	60 days	30 days	60 days
P_1	BALB × BALB	59	10.5±.35	18.6±.31	4.2	5.8	73	10.3±.20	15.9±.24	3.2	4.2
	C57 × C57	96	9.8±.35	18.5±.29	11.6	8.3	84	8.9±.33	15.5±.28	9.5	6.8
F_1	BALB × C57	43	10.7±.40	21.4±.27	6.9	3.2	51	10.4±.32	18.4±.31	5.3	5.0
	C57 × BALB	53	10.8±.40	21.5±.27	8.4	3.9	56	10.0±.24	17.7±.32	3.3	5.9
F_2	F_1 × F_1	73	12.2±.24	20.7±.27	4.3	5.4	73	11.8±.22	18.6±.22	3.5	3.6
	F_1 × F_1	45	11.7±.38	21.5±.27	6.4	3.2	46	10.9±.33	18.0±.30	5.1	4.2
	F_1 × F_1	49	11.9±.33	21.0±.25	5.3	3.2	45	10.6±.31	18.0±.25	4.4	2.9
	F_1 × F_1	55	11.4±.40	20.5±.25	9.0	3.5	53	10.6±.29	18.2±.33	4.4	5.8
	F_1 × F_1	66	12.5±.27	20.5±.29	5.0	5.7	83	11.2±.20	18.1±.23	3.5	4.4
B.C.$_1$	F_1 × C57	54	11.1±.29	20.2±.32	4.6	5.7	50	11.1±.24	17.3±.22	2.9	2.5
	C57 × F_1	38	9.7±.48	19.9±.33	8.6	4.1	52	8.7±.25	16.9±.24	3.2	2.9
	F_1 × BALB	80	11.7±.32	20.4±.26	8.5	5.8	86	10.7±.30	17.9±.20	7.8	3.4
	BALB × F_1	27	10.9±.68	20.6±.37	12.1	3.8	23	9.4±.53	17.3±.36	6.2	3.1
B.C.$_2$	C57 × B.C.	85	10.6±.34	19.6±.24	9.9	4.8	79	10.1±.23	16.6±.19	4.2	2.9
	B.C. × C57	37	11.2±.55	19.5±.34	10.9	4.4	39	9.5±.52	16.7±.31	10.5	3.7
	BALB × B.C.	58	11.7±.44	20.1±.30	9.9	5.3	64	11.2±.27	17.3±.24	4.6	3.8
	B.C. × BALB	44	13.2±.32	21.3±.34	4.6	5.1	55	11.5±.37	17.2±.31	7.8	5.3

female offspring from a C57 mother are 0.71 g lighter than those of identical genotype from the BALB mother. When the F_1 and P_1 offspring borne in the same female are compared, the differences are $2.78 \pm .41$ and $2.99 \pm .39$ for males, and $2.51 \pm .39$ and $2.19 \pm .43$ for females borne in C57 and BALB mothers respectively. Thus with the maternal environment constant, the hybrid genotype is 15% larger than the homozygous inbred genotype.

In the five lots of F_2 mice, 2 of the 10 comparisons between the mean weights of males give differences ($0.98 \pm .39$ and $0.97 \pm .36$) which are significant at the 5% level. In the females, none of the 10 differences are significant. When the F_2 is compared with the F_1, it is found that the males from the last two F_2 lots are significantly smaller than the F_1 males, while the males of the other three lots are the same weight or slightly but not significantly lighter. In the females there is no significant difference between the weights at 60 days of the two generations.

In the backcross there is no difference between reciprocal crosses, nor is there any difference in weight between the backcrosses which involve C57, and those involving BALB. The male weights decline slightly but not significantly from those of the F_2, and they are significantly lighter than the F_1 males by about 10%. Thus two thirds of the gain in the F_1 has been lost by the time of the first backcross. The females are significantly smaller than both the F_1 and F_2 females; they also show a loss of the same magnitude as the males.

In the double backcross the reciprocal crosses involving C57 have almost identical mean weights, while the reciprocal crosses with BALB give identical weights for females, but significantly different ($1.20 \pm .45$) weights for males; the males out of backcross females sired by BALB males being the larger. This generation is interesting because the crosses involving C57 continue the decreasing weight trend shown by each generation after the F_1, whereas the crosses with BALB produce males whose weights are similar to the males of the F_2 and backcross. The females from backcross $\times$ BALB are similar to those from $F_1 \times$ BALB, and are significantly smaller than the F_2 females. The fact that in both single and double backcrosses those involving BALB produce larger young than did similar crosses with C57, indicates that the slight size difference in the original parents was the result of different size alleles.

Distribution of the Variances

By the use of the variances listed in Table IV, we can assess the relative effects of genotype and environment on body size. For instance, a comparison of the variances at 30 days with the corresponding variances at 60 days, shows the effect of the maternal environment upon a particular genotype. At 30 days we are measuring what each genotype can do with the food provided by the nursing mother, whereas at 60 days we are measuring the effect of food ad libitum plus any effects which have carried over from the nursing period. Examination of the table shows that 24 out of 34 times the variance of the

30-day-old mice is higher than that of the comparable 60-day mice. It is not to be expected that all genotypes will display the same magnitude of decreased variability at 60 days, therefore it is worthwhile examining these differences to find out if they fall into an established pattern based on maternity, genotype, or sex. Dealing with the 10 exceptions first, we find that 5 of these are trivial, being less than 0.9 in the P_1. Both male and female BALB show an increase in variability at 60 days, whereas C57 shows an appreciable decrease. It should be noted that even at 60 days the C57 variance is twice as large as that of BALB. These findings are consistent with those noted under the discussion of litter size; BALB loses more young, and since these are usually the runts, this decreases the variance. The BALB strain is also more affected by diet and therefore shows less uniformity than C57 in the 30–60-day period. It is interesting to note that 90% of the variance at 30 days is between litters, and that it drops to 82% by 60 days. Thus most of the variability does not come from exceptionally large or small individual mice, but rather from some litters which do exceptionally well and others which do extremely poorly. The variance at 60 days shows a similar distribution for between and within litters; furthermore, the litters which were smaller than average at 30 days are still below average at 60 days, and vice versa. So in the parental stocks the effects of the maternal environment have a strong influence on the 60-day weight.

The F_1 males in the reciprocal crosses both show more than a 50% decrease in variance between the 30- and 60-day periods. The females, which in this generation are less variable than the males, either show no decrease between 30 and 60 days, or show an increase. This is a result of the unsettling effect on weight of the early sexual maturity of this generation. In the five F_2 groups there are six cases where the 60-day variance increases over the 30-day one, but in each case the increase was either trivial or the corresponding 30-day variance was smaller than usual. The "M" test of Rao (6, p. 228) reveals that there is significant heterogeneity (chi square 25.5 for d.f.9) indicating that the variance at 30 days is significantly larger than the variance at 60 days.

Males and females do not mature at the same rate in the two inbred lines; the weight differential appears earlier in the C57 line than it does in the BALB. Therefore it is interesting to compare the variance of the males with those of the corresponding groups of females. At 30 days the variance for the females is lower in 16 cases out of the 17 given in Table IV, while at 60 days there are 12 cases in which the females have a lower variance than the males. In all cases there is less difference between the 60-day variances than there is between the 30-day ones. It appears that dissimilarity in variance is at least partly explained by the two sexes being unlike in their rates of maturity. This point receives confirmation from the fact that in the F_1 the females mature faster than usual, and at the same time there is an increase in the 60-day variance; the females having a larger variance than the males of the

same age and generation. The use of a sex-adjusted weight with a single variance estimate is not permissible at 30 days, while at 60 days, such values should be used with caution.

Turning next to a comparison of the variances of the different generations, we find that at 30 days the variances fluctuate widely; the largest and smallest variances are not found in the generations where they are expected. The largest variances occur in the P_1, F_1, and backcrosses, while the smallest variances are found in the F_2. This is similar to the results of Chai (2), who also found that the standard deviations of the F_1 were larger than those of the P_1's in spite of the expected homeostatic effect. It is interesting to note that the C57 strain has a much higher variance than BALB, the coefficients of variability for males being 18.6% for C57 as opposed to 10.7% for BALB. On the other hand, in the backcross generation the cross to C57 has a much lower variance than the backcross to BALB. These findings indicate that much of the variation in 30-day weights is brought about by the maternal factors of the uterine and maternal environment, consequently the large variances of the P_1, F_1, and some backcrosses reflect the poor maternal environment of the inbred lines. If this is so, then part or all of this effect should be eliminated by the time the mice are 60 days old. This is what happens: the variances for 60-day-old mice are essentially the same for all generations indicating that the maternal effects have been largely eliminated.

Tests indicate that the variances in the P_1 generation are significantly unlike at each comparison; even at 60 days the C57 females have a higher variance than does BALB (χ^2 4.64, P .03). In the F_1 the variances of the reciprocal crosses are significantly different at 30 days for both males and females, but by 60 days the chi squares of 0.23 and 0.89 indicate that the variances are homogeneous. The higher variance of the males occurs when C57 is the mother, but the reverse is true in the females. It is interesting to note in this connection that at 30 days 91% of the variance is between litters, but by 60 days the proportion has dropped to 66%.

The F_2 is listed in Table IV as five family groups of almost equal size. The variances of the males are much larger than those of the females, and the five groups are not uniform, the chi square for heterogeneity being 10.18 (P .04) at 30 days and 10.45 (P .04) at 60 days. The variances of the five groups of females, on the other hand, are homogeneous with chi squares of 3.43 and 7.0 (P .5 and .14). The reason for the heterogeneity in the males is group 4 in which the males showed excessive variability, the variances falling into two groups, whereas in the females there is a wider range of variances but no grouping.

Effect of Inbreeding on Body Size

The large and small strains used in this experiment were obtained from MacArthur (5), who used mass selection based on large populations to develop these strains. After the first 30 generations, brother × sister mating was used in an attempt to produce homozygous lines for size-inheritance studies.

The details on the number of mice used, and the weights and variances of the mice in the first 30 generations are given in MacArthur (4 and 5). For the inbreeding experiment six lines of small body size and six lines of large body size were selected, and in each generation of each line four breeding cages were set up. In the large strain, the males and females which weighed the most at 60 days were selected as parents of the next generation, the average selection rate being one kept out of eight mice weighed. In the small strain, those weighing the least were selected, the average selection rate being one kept out of every six raised to 60 days of age. Several of the lines proved to be poor breeders, and it was impossible to maintain the experimental structure throughout the 20 generations; therefore no detailed statistical analysis is given.

The results are presented in Fig. 1 which shows the mean weight of both females and males in each 10th generation, along with the range displayed by the 60-day weights. This figure shows that the first 20 generations of mass selection produced the maximum increase or decrease in the selected lines,

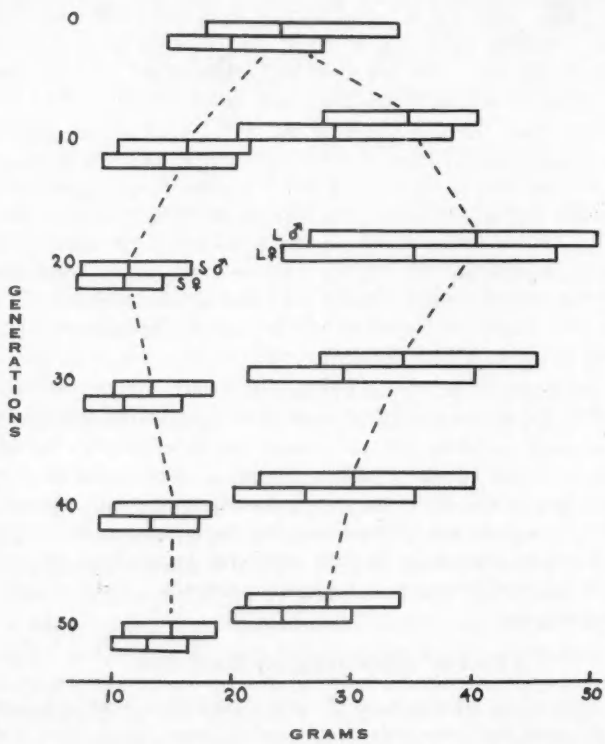


FIG. 1. The mean and range of 60-day weights for male and female mice for 50 generations of selection.

and that even with mass selection it was impossible to increase or even maintain the selected results. The subsequent inbreeding brought the two lines closer together by increasing the weights of the small line and decreasing the weight of the large line. The decreased weights of the strain with large body size are easily explained by assuming that their large weights were the result of heterozygosity, and that enforced homozygosity led to a decrease in weight. A similar explanation will not hold for the small strain since in this case the weight has increased with homozygosity instead of decreasing. Close inspection of the breeding records indicate that the smallest mice (9 and 10 g) of the small strain were poor breeders, and that when they did have a litter they seldom raised it; thus it was only the heaviest of the selected small that perpetuated the strain, and this counter selection raised the body size concurrent with the inbreeding. It is interesting to note that the variance in these lines did not decrease when the mice were inbred—this is similar to the results obtained in previous experiments (Butler (1)). It is apparent that in these experiments, decrease in the genetic component of variance is obtained only at the expense of an increase in the environmental component.

References

1. Butler, L. A study of size inheritance in the house mouse. Analysis of five preliminary crosses. Can. J. Zool. **30**, 154–171 (1952).
2. Chai, C. K. Analysis of quantitative inheritance of body size in mice. I. Hybridization and maternal influence. Genetics, **41**, 157–164 (1956).
3. Fenton, P. F. and Cowgill, G. R. The nutrition of the mouse. 1. A difference in riboflavin requirements of two highly inbred strains. J. Nutrition, **34**, 273–283 (1947).
4. MacArthur, J. W. Genetics of body size and related characters. I. Selecting small and large races of the laboratory mouse. Am. Naturalist, **78**, 142–157 (1944).
5. MacArthur, J. W. Selection for small and large body size in the house mouse. Genetics, **34**, 194–209 (1949).
6. Rao, C. R. Advanced statistical methods in biometric research. John Wiley & Sons, Inc., New York. 1952.
7. Searl, A. G. Genetical studies on the skeleton of the mouse. XI. Causes of skeletal variation within pure lines. J. Genetics, **52**, 68–102 (1954).

CONTENTS OF VOLUME 36

CORRECTION

Volume 36, page 307. The following information should be inserted in Table I between the species *Eriosoma lanigerum* (Hausm.) and *Rhopalosiphum maidis* (Fitch), under *Aphididae*:

Macrosiphoniella millefolii (DeGeer)	*Achillea millefolium* L.	17/8/57	+			
Macrosiphum solanifolii (Ashm.)	*S. tuberosum* L.	10/7/56	+	+	+	
Macrosiphum solanifolii (Ashm.)	*Rosa* sp.	12/7/57	−			
Melaphis rhois (Fitch)	*Rhus typhina* L.	18/8/56		−		
Myzus cerasi (Fab.)	*Prunus pennsylvanica* L.	9/9/56	−	−	−	−
Myzus persicae (Sulz.)	*S. tuberosum* L.	−/7/55 and −/7/56	+		+	
Myzus persicae (Sulz.)	*Brassica napobrassica* Mill.	−/8/56	+	+	+	
Periphyllus negundinis (Thomas)	*Acer negundo* L.	11/8/56	+			
Phorodon humuli (Schrank)	*Prunus nigra* L.	12/6/56	+	−	+	−
Phorodon humuli (Schrank)	*Humulus* sp.	12/8/57	−			
Prociphilus tessellata (Fitch)	*Acer negundo* L.	10/9/57		−		
(*Pterocomma populifoliae* near this species (Fitch))	*Salix* sp.	8/8/57	+			
Pterocomma smithiae (Monell)	*Salix* sp.	8/8/57	+			

CANADIAN JOURNAL OF ZOOLOGY
Notes to Contributors

Manuscripts

(i) ***General***

Manuscripts, in English or French, should be typewritten, double spaced, on paper 8½ × 11 in. **The original and one copy are to be submitted.** Tables and captions for the figures should be placed at the end of the manuscript. Every sheet of the manuscript should be numbered.

Style, arrangement, spelling, and abbreviations should conform to the usage of recent numbers of this journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed.

Manuscripts and illustrations should be carefully checked before they are submitted. Authors will be charged for unnecessary deviations from the usual format and for changes made in the proof that are considered excessive or unnecessary.

(ii) ***Abstract***

An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required, except in Notes.

(iii) ***References***

References should be listed **alphabetically by authors' names,** numbered, and typed after the text. The form of the citations should be that used in this journal; in references to papers in periodicals, titles should be given and inclusive page numbers are required. The names of periodicals should be abbreviated in the form given in the most recent *List of Periodicals Abstracted by Chemical Abstracts.* All citations should be checked with the original articles, and each one referred to in the text by the key number.

(iv) ***Tables***

Tables should be numbered in roman numerals and each table referred to in the text. Titles should always be given but should be brief; column headings should be brief and descriptive matter in the tables confined to a minimum. Vertical rules should not be used. Numerous small tables should be avoided.

Illustrations

(i) ***General***

All figures (including each figure of the plates) should be numbered consecutively from 1 up, in arabic numerals, and each figure should be referred to in the text. The author's name, title of the paper, and figure number should be written in the lower left-hand corner of the sheets on which the illustrations appear. Captions should not be written on the illustrations (see Manuscripts (i)).

(ii) ***Line drawings***

Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in blue only; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in green, yellow, or red should not be used. All lines should be of sufficient thickness to reproduce well. Decimal points, periods, and stippled dots should be solid black circles large enough to be reduced if necessary. Letters and numerals should be neatly made, preferably with a stencil (**do NOT use typewriting**), and be of such size that the smallest lettering will be not less than 1 mm. high when reproduced in a cut 3 in. wide.

Many drawings are made too large; originals should not be more than 2 or 3 times the size of the desired reproduction. Wherever possible two or more drawings should be grouped to reduce the number of cuts required. In such groups of drawings, or in large drawings, full use of the space available should be made by making the ratio of height to width conform to that of a journal page (4¾ × 7½ in.); however, allowance must be made for the captions.

The original drawings and one set of clear copies (e.g. small photographs) are to be submitted.

(iii) ***Photographs***

Prints should be made on glossy paper, with strong contrasts. They should be trimmed so that essential features only are shown and mounted carefully, with rubber cement, on white cardboard, with no space between them. In mounting, full use of the space available should be made to reduce the number of cuts required (see Illustrations (ii)). Photographs or groups of photographs should not be more than 2 or 3 times the size of the desired reproduction.

Photographs are to be submitted in duplicate; if they are to be reproduced in groups one set should be mounted, the duplicate set unmounted.

Reprints

A total of 50 reprints of each paper, without covers, are supplied free. Additional reprints, with or without covers, may be purchased at the time of publication.

Charges for reprints are based on the number of printed pages, which may be calculated approximately by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½ × 11 in.) and including the space occupied by illustrations. Prices and instructions for ordering reprints are sent out with the galley proof.

Any reprints required in addition to those requested on the author's reprint requisition form must be ordered officially as soon as the paper has been accepted for publication.

Contents